From the DEPARTMENT OF MOLECULAR MEDICINE AND SURGERY
Karolinska Institutet, Stockholm, Sweden

TRANSLATIONAL STUDIES ON BIPOLAR DISORDER AND ANOREXIA NERVOSA

Vincent Millischer

Stockholm 2020

Cover image: The building of Västerbron, Stockholm 1934.
Drawn by Irene Gutierrez Perez after a photograph by Emil Heilborn (1900-2003)

Published by Karolinska Institutet
Printed by US-AB
ISBN 978-91-7831-705-9

Translational Studies on Bipolar Disorder and Anorexia Nervosa

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Vincent Millischer

Principal Supervisor:
Prof. Martin Schalling
Karolinska Institutet
Dep. of Molecular Medicine and Surgery

Co-supervisors:
Assoc. Prof. Catharina Lavebratt
Karolinska Institutet
Dep. of Molecular Medicine and Surgery

Prof. Sophie Erhardt
Karolinska Institutet
Dep. of Physiology and Pharmacology

Dr. J. Carlos Villaescusa
Karolinska Institutet
Dep. of Molecular Medicine and Surgery

Prof. Robert Schwarcz
University of Maryland School of Medicine
Maryland Psychiatric Research Center

Opponent:
Prof. Elisabeth Binder
Max Planck Institute of Psychiatry
Munich

Examination Board:
Assoc. Prof. Janet Cunningham
University of Uppsala
Dep. for Neuroscience

Prof. Niklas Dahl
University of Uppsala
Dep. of Immunology, Genetics and Pathology

Assoc. Prof. Lars Ståhle
Karolinska Institutet &
Karolinska University Hospital

Abstract

Translational medicine aims at closing the gap between basic and clinical sciences in an integrative way. Psychiatry is one of the few medical specialties in which diagnosis is primarily based on clinical observation and all mental disorders are defined by abnormal behaviors and cognitions. The lack of biomarkers supporting diagnostic and therapeutic procedures has been a challenge in psychiatry. A better biological understanding is needed to move the field forward, it will enhance diagnostics and treatment, while reducing the stigma that surrounds mental disorders that are so poorly understood.

Over the last years, advances in fundamental sciences like genetics and neuroscience have made it clear that there is shared biology between many psychiatric disorders and that integration of methods might lead to new understandings.

The studies presented in this thesis focus on bipolar disorder (BD) and anorexia nervosa (AN), both severe mental disorders with high suicide rates, high heritability, and both lacking in biological understanding. BD, formerly known as manic-depressive disorder, is a mood disorder, characterized by manic or hypomanic episodes, often in combination with depressive episodes. AN is an eating disorder characterized by severe weight loss together with pathological behaviors.

This thesis includes five main studies on the biology underlying these disorders, based on large, well characterized cohorts, covering several methods, including genetic, imaging and protein markers, as well as preliminary data on the establishment of *in vitro* models.

Specifically, in **study I**, we attempted to replicate previously published findings on the association between subphenotypes of bipolar disorder and genetic variations in the *AKT1* gene. Using frequentist and Bayesian approaches, as well as publicly available results from genome-wide association studies (GWAS), we were able to reject previously proposed associations.

In **study II**, we explored the effects of genetic variations in genes involved in glutamate regulation on glutamate levels in two brain regions and their associations with other phenotypes. We found that the minor allele of rs3812778/rs3829280 in the 5'-untranslated region of *SLC1A2*, coding for a glutamate transporter, is associated (1) with increased glutamate levels in the anterior cingulate cortex, (2) with increased expression levels, in several brain regions, of the transmembrane receptor gene *CD44*, which is implicated in inflammation and brain development, as well as (3) with an increased risk for rapid-cycling in bipolar disorder, potentially linking *CD44*/*SLC1A2* to a more severe phenotype of BD.

In **study III**, we investigated the effects of clinical and genetic parameters on lithium pharmacokinetics in order to better understand lithium biology and improve lithium dose prediction models for bipolar patients, using the ratio between serum lithium and daily lithium intake, as outcome. We were able to confirm the association of several clinical predictors. Although no genome-wide significant locus was found, we report that genetic variation is important and might influence the outcome. Finally, based on the results obtained in the study, we developed a prediction algorithm that can be tested in the clinic.

In **study IV**, we investigated the involvement of neuronal degeneration in AN by studying neurofilament light chain (NfL), a known marker of neurodegeneration, in a case-control setting and found increased levels of NfL in patients with active AN in two different cohorts.

In **study V**, we studied the involvement of inflammation in AN, using a panel of 92 inflammatory markers in a case-control setting and report an aberrant inflammatory profile in patients with active AN, but not in patients that have recovered from AN.

These studies exemplify possible approaches that can be taken in translational psychiatry. The integration of clinical, technical and analytical approaches illustrates important learning outcomes for an aspiring clinical scientist in psychiatry.

List of publications

1. **Millischer V**, Matheson GJ, Martinsson L, Römer Ek I, Schalling M, Lavebratt C, Backlund L. **AKT1 and genetic vulnerability to Bipolar Disordery.** *Psychiatry Research (2019), in press.*

2. Veldic M*, **Millischer V***, Port JD, Ho AMC, Jia YF, Geske JR, Biernacka JM, Backlund L, McElroy SL, Bond DJ, Villaescusa JC, Skime M, Choi DS, Lavebratt C, Schalling M, Frye MA (2019). **Genetic variant in *SLC1A2* is associated with elevated anterior cingulate cortex glutamate and lifetime history of rapid cycling.** *Translational Psychiatry (2019) 9, 149.* *Equal contribution.

3. **Millischer V**, Matheson GJ†, Bergen S†, Ponzer P, Jagiello K, Stenvinkel P, Lindholm B, Martinsson L, Landén M, Backlund L, Lavebratt C, Schalling M. **Identification of clinical and genomic factors in lithium pharmacokinetics.** *Manuscript.* †Equal contribution.

4. Nilsson IAK, **Millischer V***, Karrenbauer VD*, Juréus A, Salehi AM, Norring C, von Hausswolff-Juhlin Y, Schalling S, Blennow K, Bulik CM, Zetterberg H, Landén M. **Plasma neurofilament light chain concentration is increased in anorexia nervosa**. *Translational Psychiatry (2019) 9, 180.* *Equal contribution.

5. **Millischer V***, Nilsson IAK*, Götesson A, Bulik CB, Schalling M, Landén M. **Anorexia nervosa is associated with an aberrant inflammatory profile**. *Manuscript.* *Equal contribution.

List of additional publications

Yang LL, **Millischer V**, Rodin S, MacFabe D, Villaescusa JC, Lavebratt C. **Enteric short-chain fatty acids promote proliferation of human neural progenitor cells**. *Journal of Neurochemistry (2019), in press.*

Blacker CJ, **Millischer V**, Webb LM, Ho AMC, Schalling M, Frye MA, Veldic M. **EAAT2 as a research target in bipolar disorder and unipolar depression: a systematic review.** *Molecular Neuropsychiatry (2019), Online First.*

Ho AMC, Winham SJ, Armasu SM, Blacker CJ, **Millischer V**, Lavebratt C, Overholser JC, Jurjus GJ, Dieter L, Mahajan G, Rajkowska G, Vallender E, Stockmeier CA, Robertson KD, Frye MA, Choi DS, Veldic M. **Genome-wide DNA methylomic differences between dorsolateral prefrontal and temporal pole cortices of bipolar disorder**. *Journal of Psychiatr Research (2019), 117, 45-54.*

Kumar P, Efstathopoulos P, **Millischer V**, Olsson E, Wei Y, Brüstle O, Schalling S, Villaescusa JC, Ösby U, Lavebratt C. **Mitochondrial DNA copy number is associated with psychosis severity and anti-psychotic treatment**. *Scientific Reports (2018), 8 (1), 12743.*

Almas A, Forsell Y, **Millischer V**, Möller J, Lavebratt C. **Association of Catechol-O-methyltransferase with future risk of cardiovascular disease in depressed individuals - a Swedish population-based cohort study.** *BMC Med Genet (2018) 19 (1), 126.*

Millischer V, Erhardt S, Ekblom Ö, Forsell Y, Lavebratt C. **Twelve-week physical exercise does not have a long-lasting effect on kynurenines in plasma of depressed patients**. *Neuropsychiatr Disease and Treatment (2017), 13, 967-972.*

Rahman MS, **Millischer V**, Zeebari Z, Lavebratt C. **BDNF Val66Met and childhood adversity on response to physical exercise and internet-basded cognitive behavioural therapy in depressed Swedish adults.** *Journal of Psychiatr Research (2017), 93, 50-58.*

Kumar P, **Millischer V**, Villaescusa JC, Nilsson IAK, Östenson CG, Schalling M, Ösby U, Lavebratt C. **Plasma GDF15 level is elevated in psychosis and inversely correlated with severity**. *Scientific Reports (2017), 7 (1), 7906.*

Contents

Abbreviations

Abbreviation	Term
ACC	Anterior cingulate cortex
AN	Anorexia Nervosa
AN-REC	Recovered AN
ANGI-SE	Swedish Anorexia Nervosa Genetics Initiative
BD	Bipolar Disorder
BDNF	Brain-derived neurotrophic factor
BF	Bayes factor
BMI	Body mass index
CI	Confidence interval
CNS	Central nervous system
ConLiGen	International Consortium on Lithium Genetics
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DLI	Daily lithium intake
DMEM	Dulbecco's modified Eagle Medium
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion tensor imaging
EAAT	Excitatory amino acid transporter
eGFR	Estimated glomerular filtration rate
EHR	Electronic health record
eQTL	Expression quantitative trait loci
FBS	Fetal bovie serum
fMRI	Functional MRI
FTD	Frontotemporal dementia
GABA	Gamma-aminobutyric acid
GAM	Generalised additive model
Glx	Glutamate + Glutamine (in MRS)
GM	Grey matter
GSK3	Glucogen Synthase Kinase 3
GWAS	Genome-wide association study

hES	Huma embryonic stem cells
HPA	Hypothalamic–pituitary–adrenal
ICD	International Classification of Diseases
IGF	Insulin-like growth factors
IL	Interleukin
iPSC	induced pluripotent stem cells
LD	Linkage disequilibrium
LME	Linear mixed-effects model
LOO CV	Leave-one-out cross validation
MAF	Minor allele frequency
MDD	Major depressive disorder
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MT	Metallothionein
NEAA	Non-essential amino acids
NfL	Neurofilament light chain
NSAID	Nonsteroidal anti-inflammatory drug
NSC	Neural stem cells
OCD	Obsessive-compulsive disorder
OLS	Ordinary least squares
OOS CV	Out-of-sample cross validation
PCR	Polymerase chain reaction
PEA	Proximity extension assay
PET	Positron emission tomography
PFC	Prefrontal cortex
PGC	Psychiatric Genomics Consortium
PhD	Doctor of Philosophy
PI	Prediction Interval
PI3K	Phosphoinositide 3-kinase
PLO	Poly-L-ornithine
PRS	Polygenic risk score
RAAS	Renin-angiotensin-aldosterone system
RDoC	Research Domain Criteria
RMSE	Root-mean-square error
SCÄ	Swedish center for eating disorders
SCID	Structured Clinical Interview for the DSM-IV
SL	Serum lithium
SNP	Single nucleotide polymorphism
TNF	Tumor necrosis factor
UTR	Untranslated region
WHO	World Health Organization
WM	White matter

Chapter 1

Introduction

1.1 Translational Psychiatry

1.1.1 *"A bridge to somewhere"?*

Translational medicine is the research area that aims at closing the gap that exists between basic and clinical sciences, in an integrative way. While clinical questions can become scientific hypotheses, novel biological results can be adapted to be used in clinics. This method, often referred to as *bench-to-bedside* or *bed-to-bench-to-bedside*, has been widely successful in somatic medicine.

Psychiatry is one of the few medical specialties in which diagnosis is primarily based on clinical observation, as all mental disorders are defined by abnormal behaviors and cognitions. This sets it apart from other specialties that are able to rely on lab tests, imaging methods and other more objective measurements that allow for a more precise diagnostic. The lack of biomarkers supporting diagnostic and therapeutic procedures has been a challenge in psychiatry. A better biological understanding is needed to move psychiatry forward, it will enhance diagnostics and treatment, while reducing the stigma that surrounds mental disorders that are so poorly understood.

Over the last years, major advances have been made, both in neuroscience and genetics. Although they have not yet been translated into direct clinical application in psychiatry, they have led to a better understanding of some of the biology. It has become clear that there is shared biology between many psychiatric disorders[1] and that overcoming classical definitions and analyzing disorders together can lead to new insights[2,3]. These studies have also provided examples of improved research methodology. The integration of cohorts and methods in big international consortia have led to robust and reproducible results and made it possible to capture the complexity of the disorders and analyze gene-environment interaction in new ways[4].

However big the gap, it appears that a bridge is currently being built. Nine years ago, Tomas Insel wrote a guest editorial about translational psychiatry entitled "A bridge to somewhere"[5]. In a way, this is the feeling I personally have as an early career scientist. Facing the complexity of these disorders and the current advances made, it feels like being a small worker on a huge bridge building project. The other end might not be in sight yet, but it feels that the direction is right.

In this thesis, I have used several approaches, worked on different methods and disorders, focusing on bipolar disorder and anorexia nervosa. Although this was not the plan from the beginning, I ended up participating in the building of not one but several bridges. I hope it ends up as an asset.

1.1.2 Diagnostic criteria in psychiatry

Diagnosis of psychiatric disorders is based on clinical observation and the description of symptoms. Two major diagnostic manuals are used by clinicians for the diagnosis of psychiatric disorders, chapter F in the International Classification of Diseases (ICD) published by the World Health Organization (WHO), currently in its tenth edition[6], and the Diagnostic and Statistical Manual of Mental Disorders (DSM) published by the American Psychiatric Association, currently in its fifth edition[7]. The introduction of these manuals has had a big influence in harmonizing psychiatric diagnoses all around the globe, leading to an increased reliability of diagnoses. The DSM is the main manual used by clinicians in the USA, as well as by many researchers internationally. It has however been criticized for its lack of validity, as diagnosis is mainly based on symptomatology and not, as in many other medical specialties, on more objective biological measures[8]. The authors of the DSM are aware of these shortcomings, but argue in the introduction that "*past science was not mature enough to yield fully validated diagnoses*" and "*speculative results do not belong in an official nosology*"[7]. The splitting up of the section on affective disorders into a section on major depressive disorder (MDD) and one on bipolar disorder (BD) is however, partially, based on biological findings, as the section on BDs is placed between the sections on Psychotic Disorders and Depressive Disorders "*in recognition of their place as a bridge between the two diagnostic classes in terms of symptomatology, family history and genetics*".[7]

These classifications are primarily designed for clinical use and disease categories are not as homogenous as in other medical fields. The National Institute of Mental Health has therefore developed the Research Domain Criteria (RDoC) to find new ways of studying mental disorders, developed around psychological constructs, which can be analyzed at different levels of information, going from genes to behavior[9].

Another approach is to decompose psychiatric diagnoses into endophenotypes to reduce the heterogeneity. These are defined as a set of "*neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive or neuropsychological*" features that would

allow more homogeneous grouping and facilitate genetic and biological understanding of psychiatric disorder[10]. This is the approach we have chosen in several of our studies, including the analysis of endophenotypes (e.g. psychosis, rapid cycling, lithium response) to create more homogenous groups.

1.1.3 Biomarkers in psychiatry

Currently, the use of biomarkers in confirming diagnoses of psychiatric disorders is limited and laboratory tests are mostly used to exclude organic causes of a disorder. However, it is widely understood that objective measurements and in particular biomarkers are needed to address the problem of heterogeneity in psychiatric disorders. Biomarkers are "*objectively measured and evaluated indicators of normal biological processes, pathogenic processes or pharmacologic response to therapeutic intervention*"[11]. Biomarkers can therefore be of several types, e.g. diagnostic, predictive, prognostic[12]. Development of biomarkers is tightly linked with a better understanding of the biology of psychiatric disorders, and advances in both fields will most probably go hand in hand.

1.1.3.1 Genetics in psychiatry

Many psychiatric disorders have a high heritability, and genetic studies have been quintessential in driving the understanding of psychiatric disorders forward over the last 25 years. Initially, so-called *linkage studies* focused on a few large families to identify rare gene variants conferring a high degree of disease risk. With increasing knowledge of the human genome and the common genetic variability, and the technological possibilities to easily genotype bigger cohorts, the focus was shifted to *association studies*. These are based on the correlation between disease status and genetic variation in cases versus unrelated controls and research using this approach has been extremely prolific. This method has however been criticized, as results can often not be replicated[13,14], and most results cannot be confirmed by meta-analyses. Furthermore, candidate genes are often used, with a selection based on our current understanding of the disorders. The method is therefore inherently biased towards well-studied pathways.

The current state of the art method in genetics are genome-wide association studies (GWAS) (cf. 3.2.3). When sufficiently powered, GWAS yield robust and replicable results that can often even enable valid predictions in new datasets. This is particularly true in psychiatry, where the efforts of the Psychiatric Genomics Consortium (PGC) have not only fundamentally changed our understanding of psychiatric genetics, but in several ways even brought forward new understanding of pathological mechanisms underlying psychiatric disorders[15]. Furthermore, even if early GWAS studies, based on small samples, often do not find statistically significant results, it is well established that above a certain threshold,

the number of hits is linearly associated with the number of patients[15] and collecting bigger sample sizes will lead to findings, as long as the trait is partially heritable.

GWAS have today brought up genetic associations for all main psychiatric disorders, leading to a better understanding of their biology. Furthermore, polygenic risk scores (PRS) that summarize the effect sizes of multiple genetic loci have been shown to predict phenotypes in independent cohorts. Even if their explanatory power is at the moment very low, these PRS represent a possible new way stratifying patients according to risk.

1.1.3.2 Imaging biomarkers

The brain as the obvious organ affected in psychiatric disorders has been another main research target. Diverse imaging methods have been used to study different aspects of the disorders, including magnetic resonance imaging (MRI) to study structural variation, functional MRI (fMRI) to study brain activity, magnetic resonance spectroscopy (MRS) to study metabolite concentrations, diffusion tensor imaging (DTI) to study white matter connectivity and positron emission tomography (PET) to study several biological processes[16]. For a long time, studies were single-centered, included relatively few individuals and results were often inconsistent. Therefore, despite the ever-increasing number of studies, few results have been translated into clinical practice and imaging is mainly used in the diagnosis of neurocognitive disorders, like dementias[16].

However, in recent years, the creation of large consortia has led to more reliable results, in a similar fashion than genetics. In particular, the ENIGMA consortium (Enhancing Neuro Imaging Genetics by Meta-Analysis) has driven the imaging field forwards, performing multi-center mega-analyses with unified protocols that include data from thousands of patients. Working groups exist for all major psychiatric disorders. Several imaging methods have been integrated, as well as genetic data, that allows GWAS to be performed. While the MRI field is already driven by the ENIGMA consortium, other fields (e.g. PET, MRS) have yet to produce mega-analyses based on collaborative research[17].

1.1.3.3 Blood biomarkers

Markers that can be measured in peripheral blood would be among the easiest to establish in clinical practice, as they could be combined with routine testing. Although a plethora of associations has been found for almost all psychiatric disorders, no blood marker has so far been established as a clinical biomarker. This can partly be explained by methodological problems, the aforementioned heterogeneity, a lack of specificity (e.g. low-grade inflammation present in diverse conditions[18]), as well as the complexity of the disorders that might require combinations instead of single markers (cf. Teixeira et al.[19] for BD). However, the continuous research using new methodologies (cf. 'omics') might end up producing biomarkers that would allow us to differentiate and stratify

patient groups. For example, the effect of anti-inflammatory agents seen in the treatment of MDD might be enhanced if focusing only on patients presenting with increased levels of inflammation[20].

1.1.3.4 Cell models in psychiatry

To establish biomarkers, a better understanding of the biology is often necessary. Although animal models have been very important in understanding conserved neuronal molecular pathways, no rodent model fully recapitulates any psychiatric disorder defined in DSM-V. The same applies to commonly used immortalized cell lines as *in vitro* models, as they also fail to capture the full genetic complexity of psychiatric disorders. To understand the underlying molecular bases, the use of patient tissue is therefore of utmost importance.

For a long time, the only way to get patient tissue was to sample peripheral tissue (e.g. skin fibroblasts or leukocytes), or to take *post-mortem* brain biopsies. Both approaches capture the genetic background, but have intrinsic limitations. Peripheral tissue can play a role in the pathophysiology of psychiatric disorders, however the most striking differences are to be expected in the cells of the central nervous system (CNS). *Post-mortem* brain biopsies provide insight in the pathophysiological processes taking place in the CNS but typically show the end stage of the disorder, often confounded by life-long therapies and/or comorbidities[21].

With the advent of induced pluripotent stem cells (iPSCs), many of these challenges can be solved. As iPSCs can be derived from practically any peripheral cell, including fibroblasts[22] and peripheral blood[23], tissue collection only poses minor ethical problems. iPSCs can be differentiated into any cell type and protocols for neural differentiation are becoming more and more precise. It is thus now possible to obtain neurons with the genetic background of psychiatric patients. In disorders with a high heritability, these models could lead to a better understanding of the underlying pathophysiological processes and in consequence, the development of biomarkers.

In psychiatry, the first results involving iPSC were published for schizophrenia, where patient derived neurons showed decreased connectivity, synapses, spine density and expression of glutamate receptors[24]. In BD, patient derived neurons were shown to be hyperexcitable when compared to neurons from healthy controls. This phenotype was reversed by lithium treatment in neurons from lithium responders but not from non-responders[25,26].

Although several promising results have been published on iPSC models of psychiatric disorders, it is probable that many of the studies are currently underpowered and results might not replicate. Indeed, as has been shown by the advances in psychiatric genomics, there is important heterogeneity in psychiatric cohorts that can hardly be captured by current sample sizes. Also, intra-donor variation (i.e. between two iPSC lines from the same donor) is higher than often expected. Several possible ways have therefore

been suggested to overcome this problem[27]: (1) Heavily increasing the number of included patients. However, based on adequate power for post-mortem studies that has recently been estimated to be above 25,000 patients[28], this is a challenging task that will require collaborative effort[29]; (2) reducing inter-patient heterogeneity, by focusing on well-characterized subphenotypes or on carriers of rare variants; (3) studying single mutations using CRISPR-based tools.

1.2 Bipolar Disorder

1.2.1 Clinical presentation

Bipolar disorders (BD), formerly known as manic-depressive disorders, are characterized by manic or hypomanic episodes, and depressive episodes. Manic episodes are defined as periods of abnormally elevated mood, with patients being euphoric, excessively cheerful, and full of energy, sometimes described as *feeling on top of the world*. Further characteristics are a reduced need for sleep, increased self-esteem, flight of ideas, often in combination with risky behaviors. Depressive episodes are defined by a depressive mood, diminished interest in activities, sleep disturbances, loss of energy, low self-esteem[30]. Psychotic episodes can accompany both manic and depressive episodes, even though they are more common during mania[31].

Although the current diagnostic criteria as defined in DSM-V and ICD-10 are similar, small differences exist. In the DSM-V for example, a distinction between BD type 1 and BD type 2 can be found. A single life-time manic episode[1] is sufficient for a BD type 1 diagnosis, while a diagnosis of BD type 2 requires a hypomanic[2], as well as a depressive episode.

The DSM also defines several specifiers, *with anxious distress*, *with mixed features*, *with melancholic features*, *with atypical features*, *with psychotic features*, *with peripartum onset*, *with seasonal patterns*. BD patients *with rapid cycling* are individuals who have multiple (four or more) mood episodes within one year. BD patients *with psychotic features* can be defined according to mood congruency, that is, whether the content of the psychosis is in accordance with mood polarity. While the main criteria for diagnosing BD have not changed significantly between the DSM-IV and the DSM-V, the typing according to the latest episode in BD type 1 has been removed in DSM-V[32].

The ICD-10 defines hypomania (F30.0), mania without psychotic symptoms (F30.1), mania with psychotic symptoms (F30.2), but only a single bipolar diagnosis (F31), which is however subdefined according to the current affective state and the presence or absence of psychosis.

The precise diagnostic criteria defined by the DSM or ICD can be found in appendix A.1.

[1] A manic episode entails marked impairment in functioning and lasts at least one week.
[2] A hypomanic episode is less severe, (no severe impairment) and shorter (4 days or more).

1.2.2 Epidemiology

One of the most extensive epidemiological data sets regarding bipolar spectrum disorders comes from the WHO World Mental Health study, which includes data from over 60,000 individuals from 11 countries[33]. The study reports an average 12-month prevalence of approximately 0.4% for BD type 1 and 0.3% for BD type 2. The lifetime prevalence is 0.6% and 0.4%, respectively. The authors also study *subthreshold BD*, defined as the presence of at least one symptom on the screening questions for mania, but not meeting the criteria for hypomania. This form of BD has 0.8% and 1.4% 12-month and lifetime prevalence, respectively. When pooling all three forms, the USA has the highest 12-month and lifetime prevalence (2.8% and 4.4% respectively), India the lowest (0.1% and 0.1%). The mean age of onset is 18.4 for BD type 1, 20.0 for BD type 2 and 21.9 for subthreshold BD. These results are in concordance with several previous studies[34]. BD type 1 is more frequent in men, while BD type 2 is more common in women, however overall, sex does not seem to be a major risk factor[34].

About one third of patients with a lifetime history of BD experience rapid-cycling[35]. Rapid cycling can be considered a more severe form of BD, as patients do not only have many more episodes, but also a younger age of onset, higher persistence, more severe depressive episodes[35]. Up to two-thirds of patients with BD are estimated to have at least one episode of psychosis during their lifetime[36].

Seventy-five percent of all patients with one form of BD have at least one psychiatric comorbidity, but many patients have three or more disorders. Anxiety disorders (62.9%), behavioural disorders (44.8%) and substance use disorders (36.6%) are the most common comorbidities[33]. Another important problem among BD patients is suicidality with 43.4% reporting suicidal ideation, 21% planning, and 16% making suicide attempts over the last year. A recent meta-analysis reports 0.164 suicides per 100 person-years[37], meaning that approximately 3.4-5.9% of all suicide deaths occur among people with BD.

1.2.3 Pathophysiological considerations

As discussed in section 1.1.2, BD is a clinical diagnosis, currently not based on any (neuro)biological definition. This is partly due to the fact that the pathophysiology of BD is still poorly understood and no clinically useful biomarkers exist. Unfortunately, this creates a vicious circle, as the purely descriptive definition most probably pools conditions together that, although having the same clinical presentation, represent different pathological entities[38], which in turn makes the discovery of neurobiological substrates more difficult. Nevertheless, the last years have seen advances in the understanding of the disease, mainly driven by large consortia, which give hope that better definitions are possible. In this section, I will discuss the most established findings, focusing in particular on pathophysiological aspects important for the studies included in the thesis.

1.2.3.1 Genetics

BD runs in families and many patients have relatives with a history of mood or psychotic illnesses. Family history of the disorder is therefore used by clinicians to strengthen a new diagnosis.[3] Twin studies have revealed a heritability of 60-80% and a monozygotic concordance rate of 40-70%[39,40]. The relative risk for a sibling of a patient compared to risk in the general population is about eight-fold[39,40]. These estimates are among the highest for any psychiatric disorders[31]. In general, studies suggest that, like most psychiatric disorders, BD is characterized by polygenic inheritance, based on many common variants with small effect sizes[38], increasing the risk in interaction with environmental factors[31].

There is important genetic overlap with other psychiatric disorders, including schizophrenia (r=0.7), MDD (r=0.36), and obsessive-compulsive disorder (OCD) (r=0.31)[41]. Finally, there seems to be a positive genetic correlation between BD and AN (r=0.19-0.21)[41,42], even though, in the latest analysis, its p-value ($p = 2 \cdot 10^{-4}$) was just above the predetermined significance level ($\alpha = 1 \cdot 10^{-4}$)[42].

Many gene association studies have been performed before the GWAS era, focusing mainly on biologically plausible candidates like the serotonin transporter gene *SERT*, the brain derived neurotrophic factor gene *BDNF* and the catechol-o-methyl transferase gene *COMT*. Many hundreds of SNPs have been associated to BD, however, robustness of these findings is an issue, as can be exemplified by a meta-analysis which included 33 SNPs[43]. In this study, only SNPs in four genes (*BDNF*, the dopamine receptor D4 gene *DRD4*, the D-amino acid oxidase activator gene *DAOA*, and the tryptophan hydroxylase gene *TPH1* were found to be significant at a significance threshold of $\alpha = 0.05$ and none of the results remained statistically significant after correcting for multiple testing. This is further exemplified in study I of this thesis (cf. 4.1, where using a large sample, as well as public GWAS data, we propose that published associations between variants in *AKT1* and BD are less likely to be true.

The advent of large GWAS, driven by the PGC, has led to a major change in the understanding of the genetics of BD and growing patient cohorts included in the studies have brought forward robust and reproducible results. The latest published GWAS[44] is a meta-analysis of 32 cohorts from 14 countries, including a total of 20,352 cases and 31,358 control, testing for almost 10,000,000 autosomal variants with a minor allele frequency (MAF) > 1%. All variants with $p < 10^{-4}$ were then tested for association in an independent follow-up sample. This study reports 30 loci that achieved genome-wide significance, in the combined sample: these are located among others near genes involved in calcium signaling (*CACNA1C*, encoding for L-type calcium channel subunit gene), genes involved in ion transport (*SCN2A*, encoding for a sodium voltage-gated channel subunit; *SLC4A1* encoding an anion exchanger; *ANK3*, encoding ankyrin 3, involved in the localization of sodium channels), glutamate signaling (*GRIN2A*, encoding for a NMDA-receptor

[3] Personal communication from Dr. Lena Backlund

subunit), as well as other genes involved in brain physiology (*NCAN*, encoding the brain expressed extracellular matrix glycoprotein neurocan that is involved in neuronal adhesion and neurite growth[45]). Pathway analyses pointed towards an involvement of insulin and endocannaboid signaling[44].

The overlap between BD and schizophrenia is also supported by the fact that many of the reported loci have previously been associated with schizophrenia (e.g. *TRANK1*, *ITIH1*, *CACNA1C*, *NCAN*)[46]. Furthermore, a combined analysis of BD and schizophrenia revealed over a hundred loci associated with the combination of both disorders, many of them related to synaptic and neuronal biology[2]. In this analysis, only 2 loci were divergent between BD and schizophrenia. Interestingly, the PRS based on the BD cohort was able to predict psychosis in BD, while a BD PRS was associated with manic symptoms in schizophrenia.

Copy-number variants (CNV) have also been implicated in BD. Although being less common, they yield a far bigger effect size than common SNPs. A meta-analysis performed by Green and colleagues found three CNV loci (*1q21.1 duplication, 3q29 deletion, 16p11.2 duplication*) with odds ratios of 2.6-17.3[47]. These CNV are also associated with schizophrenia[48]. Overall CNV involvement in BD is less important than in schizophrenia[49].

1.2.3.2 Imaging

The Bipolar working group of the ENIGMA consortium has published several MRI studies providing evidence for changes in cortical and subcortical grey matter, as well as in white matter connectivity. Cortical grey matter was shown to be thinner in frontal, temporal and parietal regions of BD patients compared to healthy controls[50]. Furthermore, BD patients had a reduced volume of the hippocampus and thalamus, enlarged ventricles[51], as well as lower fractional anisotropy, a measure of neural connectivity, in several white matter tracts, with the biggest effects seen in the corpus callosum[52].

As mentioned in 1.1.3.2, other imaging techniques (e.g. PET, MRS) have not formed big consortia and the results of these methods are therefore best summarized through (systematic) reviews and, where possible, meta-analyses. One such result is the glutamatergic dysregulation shown in BD patients using MRS (cf. 3.3). A meta-analysis including 17 studies comparing BD patients and healthy controls reported higher levels of Glx (glutamate + glutamine) in the frontal areas and when combining all regions. Furthermore, it also found non-significant trends for increased Glx/Creatine ratio and glutamate when combining all regions[53]. A separate meta-analysis including 15 studies on frontal regions and eight studies on the anterior cingulate cortex (ACC) concluded that BD patients showed significantly elevated Glx levels in both regions[54].

There has been some effort to integrate genetic and imaging studies in order to achieve a better understanding of the biology behind BD, among others by the ENIGMA consortium. Several smaller studies, mainly based on candidate genes, have brought forward results,

but only few have been replicated. GWAS with imaging data has so far not revealed any genome-wide association for imaging findings in BD. A recent systematic review[55] however highlights several associations supported by more evidence, including (1) the association between genetic variation in *CACNA1C* and activation of the amygdala; (2) the association between genetic variations in *ANK3* and white matter structure; (3) the association between the BDNF met allele in rs6265 and smaller hippocampal volumes. However, due to several methodological limitations (e.g. sample size, correction for mood states) these results have to be interpreted with care.

1.2.3.3 Cell culture

As discussed in 1.1.3.4, *in vitro* disease modelling is a powerful tool that combines the advantages of harbouring the genetic information of patients with the possibility of mechanistically testing for effects of drug treatment or altering genes of interest. Over the years, BD has been very extensively modelled using peripheral models like lymphoblastoid cell lines and fibroblasts. These studies highlighted changes in several intracellular signaling mechanisms (e.g. calcium signaling, inositol signaling), as well as in mitochondria, oxidative stress and circadian rhythm[56]. More recently, several iPSC-based studies have provided new insights into potential disease mechanisms: Neurons differentiated from iPSC derived from BD patients were shown to be hyperexcitable, a phenotype, which can be reversed by lithium treatment[25]. Furthermore, electrophysiological characteristics were found to differentiate between lithium responders and non-responders[26]. Finally, similarly to other psychiatric disorders iPSC-based models have been proven to be interesting to follow-up on GWAS results in BD. For example, a recent study showed that iPSC derived neural progenitor cells carrying the risk allele for BD of rs9834970 near the *TRANK1* gene, had lower baseline *TRANK1* expression and that this phenotype could be rescued by treatment with valproate[57].

1.2.3.4 Further pathophysiological considerations

Neuropathological changes. While there has been many studies on neuropathological changes in BD, most of them rely on small samples, and few results have been replicated. A recent meta-analysis found support for some findings, including decreased cortical thickness in the ACC and reduced neuronal density in the amygdala, while pointing out that no pathological finding can be considered to be established "beyond reasonable doubt"[58].

Neuroendocrinology. The involvement of the hypothalamic–pituitary–adrenal (HPA) axis has for a long time been well established[59]. A recent meta-analysis showed that BD patients have higher cortisol (awakening, morning, afternoon, night) and basal adrenocorticotropic hormone levels, but found no difference in corticotropin releasing hormone[60]. Furthermore, euthymic BD patients show a flattening of the cortisol curve,

which indicates a HPA axis dysregulation[61]. BD patients also exhibit higher levels of extra-neuronal noradrenaline[62], as well as disturbed thyroid function[63].

Inflammation. Increased inflammation is well documented in BD. Several cytokines and their receptors have been shown to be elevated in BD, depending on the mood state, by several meta-analyses including C-reactive protein (CRP), Interleukin (IL) receptor 1 antagonist, IL6, soluble IL-2 receptor (sIL-2R), sIL-6R, tumor necrosis factor (TNF)-α, soluble TNF receptor type 1[64–66]. Furthermore, BD has positive genetic correlations with several immune-related conditions, including celiac disorder, psoriasis, ulcerative colitis, and Crohn's disease[67].

Circadian dysfunction. Circadian disturbances have been strongly associated with BD in all three states of the disorder (mania, depression and euthymia). Changes in many characteristics of sleep quality[68–70], delayed and irregular sleep-wake cycles[71] and abnormal daily activity[72] have been reported. These clinical characteristics are in congruence with reported disturbances in melatonin signaling[68,69,73].

Mitochondrial function. Disturbances in mitochondrial function and energy dysregulation have also been linked to BD[74]. This has been characterized among others by increased reactive oxygen species production and decreased mitochondrial complex subunits in the brain.

1.2.4 Treatment

1.2.4.1 General considerations

Although BD cannot be cured today, it is highly treatable and manageable. Treatment is based on so called *mood stabilizers*, which include lithium, anticonvulsants and some atypical antipsychotics, and up to 90% of patients achieve a substantial improvement of their condition. Unfortunately, many patients are not receiving adequate treatment, particularly in low income countries, only a minority of patients have contact with the mental health system[33].

1.2.4.2 Lithium

The alkali metal lithium was first used in 1949 to treat "psychotic excitement"[75] and has since then been established as the cheapest and most effective treatment for BD[76]. It is often prescribed for life and leads to a substantial increase in life quality. Lithium has a very small therapeutic window (0.5-1.3 mEq/L), with lower levels generally considered subtherapeutic and higher levels being toxic. Toxic effects include gastro-intestinal symptoms, drowsiness, tremor, muscle weakness at levels above 1.5 mmol/L; levels higher than 2 mmol/L can be severely toxic, leading to seizures, kidney failure, hyperthermia,

coma and delirium, and require intensive care[77]. Lithium has excellent anti-manic and anti-suicidal effects[78]. Its effects on depression are however less potent. Approximately one third of the patients responds extremely well to lithium, while one third does not respond at all[79].

1.2.4.2.1 Pharmacodynamics

The exact mechanisms of lithium are currently unknown, but many biological pathways have been suggested to play a role. On a cellular level, lithium mediates neuroprotection through inhibition of glycogen synthase kinase 3β (GSK-3β), the increase of peripheral BDNF levels and influencing oxidative metabolism and apoptosis[80,81]. It further influences several second messenger systems, including the phosphoinositide cycle, with lithium treatment leading to increases in myo-inositol levels, protein kinase C, intracellular calcium, and adenyl cyclase[80,81]. Lithium also acts on neurotransmission, including glutamate, dopamine and γ-aminobutyric acid (GABA) signaling. Finally, it influences higher order biological systems like circadian rhythm, the HPA axis, as well as the repair of gray and white matter abnormalities[80,81]. However, although many different mechanisms have been suggested, it is unlikely that any single one of them can explain the anti-manic and anti-suicidal effects of lithium alone. For example, a study comparing lithium to selective GSK-3β inhibitors in rats concluded that even if some effects could be seen with both components, the overall effects were fairly different[82].

1.2.4.2.2 Pharmacokinetics

Lithium is most commonly administered as tablets. Different lithium salts exist, e.g. carbonate, acetate, citrate, gluconate and sulfate, all have however a bioavailability of 80-100%[83]. After uptake in the proximal parts of the small intestine[84], lithium enters the blood stream as free ions. Contrary to many other drugs, lithium is not metabolized and distributes freely in the body, according to a two-compartment model[83,85]. Brain lithium concentrations are generally considered to be smaller than serum concentrations[86], with important heterogeneity within the brain[87].

Lithium is primarily eliminated through the kidneys[83]: It is filtered in the glomeruli, however, 70-80% are reabsorbed in the proximal tubules together with sodium ions[88], which can lead to increased lithium reabsorption and higher serum levels during hyponatremia[83]. The terminal elimination half-life is estimated at 20-40 h[89,90]. Several variables have been found to influence elimination and explain part of the variability, e.g. age[91], kidney function[89], body mass index (BMI)[92] and treatment duration[93]. Disorders of the kidney and the heart, as well as co-medication with sodium-depleting diuretics, antihypertensive agents and non-steroidal anti-inflammatory drugs (NSAID) have been shown to reduce lithium elimination[85].

1.2.4.2.3 Pharmacogenomics

Even if some clinical features (initial good response, positive family history of BD with a good response to lithium, BD type 1) can be used to help to predict lithium response, no generally accepted biological marker exists[94]. There is evidence that lithium response is a heritable trait, which has led to several pharmacogenomic studies trying to find genetic markers associated with lithium response. The biggest study so far, published by the International Consortium on Lithium Genetics (ConLiGen), found a single locus on chromosome 21, containing two genes for long, non-coding RNAs, associated with lithium response. The collection for a new GWAS is currently ongoing.

1.2.4.2.4 Treatment regimen

Because of its small therapeutic window, lithium dose has to be adjusted to each patient, to avoid toxic effects. Usually, lithium therapy is started by an individual titration. A low dose is given (in Sweden usually 2 tablets of lithium sulfate, i.e. 12 mmol/day) and the serum concentration is measured when the steady state is reached after approximately one week. The dose can then be adapted, and the process is repeated until the planned levels are reached[95]. The finally required dose can vary greatly. During the initiation period, which can take several weeks, the patients are not adequately treated. Unfortunately, there is no model to predict the amount a patient has to take to reach therapeutic levels. However, even if the clinical variables influencing lithium pharmacokinetics are well studied, they only explain part of the variance and the genetics behind it are poorly understood.

1.3 Anorexia Nervosa

1.3.1 Clinical manifestation

Anorexia nervosa (AN) is an eating disorder characterized by severe weight loss. While mostly achieved by fasting, other behaviors supporting low body weight can be present. These include increasing energy expenditure by excessive exercise, abuse of drugs that increase metabolism (e.g. thyroid hormone), or purging, by self-induced vomiting, abuse of laxatives, diuretics or enemas[96]. Despite the low body weight, patients present with an intense fear of gaining weight or being overweight, and are often building their self-worth on the body weight. A disturbed body image is commonly associated with the disorder[97]. Patients often do not recognize the severity of the disorder, even when the weight loss is life threatening[96].

The diagnostic criteria of DSM-V and ICD-10 (cf. A.2) are fairly similar, recognizing as main points the restriction of energy intake, low weight, fear of gaining weight, associated behaviors and disturbances in the way the body is viewed. The DSM-V

further differentiates between restricting type and binge eating/purging type[4], diagnoses defined by the clinical modification of ICD-10 (ICD-10-CM). The DSM-IV criteria also contained the absence of *three consecutive non-synthetically induced menstrual cycles* in menstruating women as a diagnostic criterion, which has been dropped in DSM-V. DSM-V on the other hand, also defines criteria for partial and full remission[32,98].

AN is usually a long-lasting illness, with a median time to remission of about 7 years for female patients[99]. It is speculated that one of the reasons behind the long duration of the illness is the transformation of the behaviors, specifically dieting, from goal directed (i.e. aiming for a low body weight) to habitual[100]. The consequences of low body weight and starvation are manifold: AN starvation is accompanied by a spectrum of secondary effects that occur either directly due to starvation or result from adaptive processes to starvation. These are often difficult to distinguish from mechanisms that drive the disorder[101].

1.3.2 Epidemiology

AN is known to be a disorder that mostly affects adolescent girls and young women. However, all ages, sexes, sexual orientations, races and ethnic groups are affected[102]. Epidemiological research is complicated by several factors, among other the generally low incidence, as well as the unwillingness of many patients to recognize the severity of the disorder and to seek treatment[96]. Furthermore, cultural aspects also influence the results. A multi-informant approach together with reliable and valid screening methods are needed to capture all aspects of the disorder and study its epidemiology[96,103].

Epidemiological studies have also struggled with the definition of AN, using either strict or broad definitions. Broader definition can be achieved by using less severe cutoffs for BMI, and are more inclusive concerning weight gain, as well as amenorrhea (when using DSM-IV criteria). Lifetime prevalence using strict definitions is about 0.5-2%[104–107], and reach up to 4.3%[106] when applying the broader definitions. Lifetime prevalence in men is estimated to be below 0.5%[107,108].

Depending on the cohort studied, the incidence rates widely differ. In Western countries, estimates range from about 7 per 100,000 person-years in primary care registries[109,110], to above 200 per 100,000 person-years in adolescent girls[105,111].

Sex is the strongest risk factor for AN, with many more women than men being affected by the disorder. Estimates go from 8:1[110], to up to 15:1[112] in adult populations. In children and adolescents, the sex distribution is less skewed[113].

Co-morbidities are very common in AN. The life-time prevalence of MDD in AN is estimated between 60-80% depending on the subphenotype[114]. Anxiety is also very prevalent, with estimates ranging from 23% to 75%[115]. Symptoms of OCD are frequent,

[4]Binging: Eating large amount of food in a short time, without necessarily being hungry.

being present in up to 40-60% of patients[116,117], even though most studies report about 25%[118]. This co-occurrence seems to be at least partly due to a shared heritability between both disorders[119]. Alcohol and drug abuse range between 13-24% and 6-18% respectively, depending on the subtype[120]. Finally, autistic traits are common in patients with AN[121], and AN and autism co-aggregate in families[122].

AN patients have an increased mortality rate compared to the general population[123], with a standardized mortality rate estimated at 5.86. Approximately one fifth of the deaths are due to suicide[124]. It is however important to note that recovery is possible, as it is estimated that up to 60% of patients will recover from AN[125,126]. However, these estimates also vary, as the definition of recovery is under debate[127]. In our studies, we used a common approach, which considers individuals as recovered when their weight has normalized and no pathological eating patterns and excessive exercise have been present for more than one year.

1.3.3 Pathophysiological considerations

For a long time the causes of AN were mainly considered to be psychological. However, in recent years, biological factors have gained importance. In particular, results from GWAS based on large cohorts have brought forward new understanding of the mechanisms behind the disorder. Nonetheless, the pathophysiology is still poorly understood. I will discuss the most established findings, as well as aspects that are most important for the studies included in the thesis.

1.3.3.1 Familial and psychological risk factors

As mentioned in section 1.3.2, the highest and best-established risk factor for AN is sex. It is now well established that social, environmental and behavioral characteristics of the family cannot be seen as solely causal for AN. Nonetheless, there is some indication that socioeconomic variables, in particular parental education, are associated with higher risk for AN[126,128].

Personality traits and cognitive styles have been consistently associated with AN, including weak central coherence[129], emotion dysregulation[130], impaired inhibitory control[131], perfectionism[132], aberrant reward sensitivity[133], low-self-esteem[134]. These traits are present before the onset of the disorder, but become more prominent during the acute stages. Some have been shown to affect the prognosis[126]. It is however important to underline that the separation between psychology and biology is difficult, as they influence each other.

1.3.3.2 Genetics

There is strong evidence that AN runs in families, with AN being 4 times more frequent in families of patients with AN than in control families[135]. Female relatives have an up to 11 times higher risk for AN than females that do not have any AN patient in the family[136]. Twin studies have estimated the heritability for narrow AN to be between 46-74%[137], the estimates for broad AN being a bit lower (29%[138]).

As for BD, genetics of AN was initially driven by linkage studies, followed by candidate gene approaches. These were based on *a priori* hypotheses, and included genes that were thought to be biologically relevant, among others *GHRL*, *MC4R*, *POMC*, *ESR1*, *ESR2*, *FTO*, encoding for Ghrelin, Melanocortin 4 receptor, Proopiomelanocortin, Estrogen Receptors 1 and 2, and Fat mass and obesity associated, respectively[139]. However, studies were often underpowered and results did not replicate[139].

The advent of GWAS has also pushed the field of AN genetics forward, even if the cohorts have historically been smaller than in other psychiatric disorders. The first genome-wide hit was published in 2017 by the Eating Disorder Working Group of the PGC (PGC-ED)[140]. This study also showed genetic correlations between AN and several other psychiatric disorders, but also metabolic traits like BMI, insulin, glucose and lipid phenotypes. In 2019, combining data from the Anorexia Nervosa Genetics Initiative (ANGI) and the PGC-ED (16,992 cases of AN and 55,525 controls), eight significant loci were published[42], highlighting the role for *MGMT*, *CADM1*, *FOXP1*, *PTPB2*. *MGMT* encodes for O-6-methylguanine-DNA methyltransferase, a DNA repair protein involved in defense against mutagenesis[141]; *CADM1* has been associated with BMI by GWAS and animal models[142]; *FOXP1* has been associated with hippocampal and striatal development, as well as with body weight in mice[143]; finally, *PTPB2* is implicated in regulation of splicing[141].

Although the estimated SNP heritability was only 11-17% and PRS analyses capture 1.7%, several genetic correlations with psychiatric disorders could be found, including OCD (r=0.45), MDD (r=0.28), anxiety disorders (r=0.25) and schizophrenia (r=0.25), reflecting observations from clinical studies. Furthermore, several metabolic and anthropometric traits negatively correlated with AN, e.g. fasting insulin (r=-0.24) and leptin (r=-0.26), type 2 diabetes (r=-0.22), fat mass (r=-0.33) and BMI (r=-0.32). Further analyses revealed that associated genes were enriched in brain tissues, and, on a cellular level, medium spiny neurons and pyramidal neurons from hippocampal CA1. Overall, the authors conclude that their result support both metabolic and psychological causes for AN.

Finally, there is some indication that epigenetic changes could contribute to AN. This is supported by a family study which found a missense mutation in the histone deacetylase 4 gene (*HDAC4*) segregating with AN, as well as reports on altered methylation patterns in the *HDAC4* locus[144].

1.3.3.3 Imaging

Neuroimaging studies have also brought forward insights into disease mechanisms of AN. Evidence shows that patients with AN have volume reductions in several brain areas, affecting both grey (GM) and white matter (WM)[145–147]. While the support for a reduction of GM is quite strong[148,149], the results on WM are generally more inconsistent[149,150]. Most of these changes reversed with weight restoration[148,151–153], even if some studies report persisting differences between AN-REC and controls[154]. One study by the ENIGMA consortium evaluating the relationship between genetic variants affecting subcortical brain volume and those affecting AN, found inverse correlation between the risk for a greater thalamus volume and risk for AN[155].

A recent meta-analysis showed a reduction of global GM in acutely ill AN patients, which normalized over time after recovery[149]. The authors report that these changes particularly affect hippocampal and cingulate regions. However, many of the considered studies were small and did not correct for age, BMI, nutritional status, and it has been suggested that the results therefore mainly reflect malnourishment[148,149]. Controlling for these factors, some studies have reported increases in grey matter volume in several areas of the brain, e.g. the orbitofrontal cortex and the insula[148].

Regarding WM, significant differences were found in patients with acute AN when compared to controls, but not after recovery[149]. Lower fractional anisotropy was found in several WM tracts, mainly in the fornix and the cingulum, pointing to an involvement of the limbic system[150].

PET studies have mainly focused on serotonin and differences in serotonin receptor in several brain areas have been shown[96]. Finally, functional MRI studies have revealed differences in activation patterns of areas involved in reward processing and cognitive control[156].

1.3.3.4 Endocrine alterations

AN is accompanied by important endocrine alterations, in particular when patients are underweight[96]. These affect almost all hypothalamic-pituary pathways[157]. Regarding the reproductive system, reduced gonadotropin-releasing-hormone signaling leads to decreased follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels and as a consequence low estrogen levels, anovulation and infertility. Puberty can also be delayed[157]. The HPA-axis is also often dysregulated, with higher cortisol levels found in patient with AN than in controls[158]. Furthermore, high growth hormone levels, low insulin-like growth factor (IGF)-I, as well as abnormal thyroid hormone levels have been described[159,160]. Abnormalities in hormones that regulate appetite have been described, mostly in line with low nutritional state, even if the signaling seems to be impaired: leptin levels that normally signal satiety, are low[161]; ghrelin levels, a hunger signal, are high[162].

Finally, endocrine alterations are also thought to be involved in a higher risk for reduced bone density and osteoporosis, often present in AN patients[163].

Overall, as most of these changes normalize with weight restoration, it is unclear if these changes are causes or consequences of the disorder.

1.3.3.5 Inflammation

The bidirectional relationship between inflammation and food intake is well established. Reduced food intake is a common symptom of sickness behaviour occurring during an increased inflammatory state[164] and several cytokines are anorexigenic (e.g. IL-1β, IL-18[165,166]). On the other hand, both obesity, as well as malnutrition and wasting have been shown to be associated with changes in the immune system: While the first is accompanied by low-grade inflammation[167], the second is marked by a weakened immune system and higher levels of infections[168].

It is now well understood that there is strong cross-talk between peripheral inflammation and the CNS[164] and that dysregulated inflammatory processes might play a role in several psychiatric disorders[169]. For example, patients with MDD have increased serum levels of proinflammatory cytokines such as IL-6, TNF-α and CRP, while the strongest genetic association for schizophrenia is in the major histocompatibility complex (MHC) locus, pointing to an involvement of the complement system. Furthermore, several psychiatric disorders have shown genetic correlations with inflammatory disorders, which means that the genetic risk of one type of disorders increases the risk for the other type[67].

Based on these facts one might speculate that AN is associated with a dysregulated immune system. However, the role of inflammation in AN is not yet well established. For example, while a bi-directional relationship for risk for eating disorders and autoimmune disorders has been found in epidemiological studies[170], on a genetic level, no positive correlation with any autoimmune disorder was reported. The only significant correlation to date is a negative genetic correlation between AN and CRP levels[67]. Furthermore, autoimmunity has also been suggested to play a role in the pathophysiology of AN, with auto-antibodies against a-melanocyte-stimulating hormone, a neuropeptide in the brain signaling satiety having been described in plasma of patients[171]. Finally, changes in several cytokines were shown between AN and healthy controls, two of which (IL-6 and TNF-α) were also significant in the most recent meta-analysis[172]. However, it is important to notice that previous studies on inflammatory markers in AN are scarce and typically based on small samples.

1.3.3.6 Cell culture

There are only few *in vitro* studies of AN. Several studies have analyzed *in vitro* cytokine production, but there are, to our knowledge, no studies using fibroblasts or lymphoblastoid

cell lines. One single study was based on iPSC from AN patients (N=7)[173]. After differentiation into a mixed neuron population, although no obvious differences between AN and controls were detected, the authors suggest *TACR1* (tachykinin receptor 1) to be associated with AN, based on pathway analyses and protein measurements.

1.3.3.7 Animal model: The *anx/anx* mouse

Several animal models for AN exist, including activity based[174], stress and diet related[175], as well as genetic models[176]. Here we are going to shortly discuss the *anx/anx* mouse, a model that has been used in our lab for many years and played an important role in the hypothesis generation for our human studies.

The *anx* mutation that these mice carry arose spontaneously in a cross from several mouse strains[177], which makes the identification of the exact mutation difficult. Homozygous mice appear healthy at birth, but start eating significantly less than their heterozygous siblings, even if they have full access to food[177]. This leads to gradual emaciation, and the animals die prematurely around week 3[177]. This mirroring of core features of the disorder has led to the mouse being extensively studied as a model for AN. The animals present with major changes affecting organs, behaviors, metabolic traits and neurotransmitter systems[176,177]. Furthermore, several disturbances in the hypothalamus have been described, including changes in the neuropeptidergic innervation, hypothalamic inflammation, as well as degeneration[176]. These were the phenotypes we tried to study in humans in study IV and V.

1.3.4 Treatment

Recovery from AN is possible: It is estimated that a majority of patients overcome the disorder, a third show improvements, while about 20% become chronic.[178]. However, only a minority of patients will ever seek treatment[126]. Re-feeding, essential in the treatment in order to increase body weight, is only the first step in the management of AN. Even if it is achieved during the admission to a specialized clinic, patients are not always able to maintain the improved nutritional status[96]. Psychotherapy therefore plays an important role and represents the treatment of choice for AN, with several treatment paradigms available. While family-based treatments have shown the best results in adolescents, there is no clear gold standard therapy for adults. There is little evidence supporting the use of pharmacotherapy, with studies on antidepressants and antipsychotics showing no or weak results on eating habits, weight and psychological comorbidities[179,180]. However, several prevention programs have shown promising results in reducing the symptoms in AN and disorder onset[96].

1.4 Research considerations

1.4.1 Bipolar disorder and anorexia nervosa

BD and AN are separate entities and this thesis does not focus on the overlap between these two disorders. There are some similarities, as mentioned previously, both being relatively common and their prevalence often underestimated by the general public. Furthermore, they are highly heritable, show a certain amount of genetic correlation, as well as overlap with similar psychiatric disorder (e.g. OCD) and their underlying biology poorly understood.

Some epidemiological evidence points towards an overlap between the eating disorders and BD, with eating disorders being common among BD patients[181,182]. However, this association seems to be mainly driven by binge-eating disorder and bulimia nervosa and only to a lesser extent by AN[181,183,184]. However, the similar comorbidity profiles (suicidality, mood instability, anxiety disorder), as well as an overlap in symptomatology (eating and weight dysregulation in BD, mood lability and cyclicity in AN)[183,185] underline the complexity of the relationship, and the need for more research[186].

1.4.2 Trait and state

Two further issues to discuss are the distinction between *trait* and *state*, as well as between *cause* and *consequence*. A trait is a more stable and enduring characteristic, while state refers to a temporal way of being, often related to a current clinical state. While the general genetic liability for BD or AN could be considered a *trait*, a current manic episode or low BMI in AN could be considered as a state[187]. It is thought that markers can be found for both traits and states. While trait markers are linked to processes that predate the clinical picture, and even potentially play a causal role, state markers are intimately linked to a clinical manifestation.

Regarding causality, it is well known that association does not imply causation. While the associations with markers that cannot change or are unidirectional (e.g. DNA) can more easily be seen as causal, causality is more complex to establish for many factors (e.g. blood or imaging markers). It is not possible to sort this out with cohort studies but more sophisticated research protocols (e.g. longitudinal studies) are needed.

1.4.3 Outlook

Overall, this thesis presents studies covering two different disorders using several methodologies. These are examples of possible research approaches that break up classical separations, and integrate different fields to take a larger view at translational research in psychiatry.

Chapter 2

Aims

The aim of this thesis was to study the biology related to bipolar disorder and anorexia nervosa. While **studies I to III** were mainly focusing on genetics of bipolar disorder, both in regards to pathophysiology and lithium treatment, **studies IV and V** aimed at exploring biomarkers of neurodegeneration and inflammation in anorexia nervosa.

The specific aims of the studies included were:

Study I: To replicate previous findings on the association between subphenotypes of bipolar disorder and genetic variations in the *AKT1* gene.

Study II: To explore the effects of genetic variations in genes involved in glutamate regulation on glutamate levels in the brain and following up the link to bipolar disorder.

Study III: To investigate the effects of clinical and genetic parameters on lithium pharmacokinetics in order to better understand lithium biology and improve lithium dose prediction models for bipolar patients.

Study IV: To investigate the involvement of neuronal degeneration in anorexia nervosa by studying neurofilament light chain (NfL) in a case-control setting.

Study V: To investigate the involvement of inflammation in anorexia nervosa, by studying a panel of 92 inflammatory markers in a case-control setting.

Chapter 3

Materials and Methods

This section includes the description of the cohorts, as well as conceptual explanations and discussions of the methods used in the thesis. More detailed descriptions of the methodologies, in particular regarding technical details, can be found in the Material and Methods section of each individual study.

3.1 Cohorts

3.1.1 Swedish bipolar cohort (Study I, II and III)

This is the main BD cohort our research group has access to and contains today more than 800 unrelated patients with a diagnosis of BD. Patients were consecutively recruited from the Unit of Affective disorders, Psychiatry Southwest, at the Karolinska University Hospital in Huddinge. The collection was started in 2003 and is still ongoing.

Symptoms were recorded according to DSM-IV by a psychiatrist specialized in BD or a trained psychiatric nurse, based on interview and electronic health records. The data collected included general demographic information, information on diagnosis (BD type 1 or type 2), age of onset, number and length of episodes and hospitalizations, affected family members, as well as history of psychosis (including mood-congruency) and rapid-cycling (Studies I & II). For patients treated with lithium (N = 599), information on lithium treatment (e.g. number of tablets, lithium concentrations, several variables possibly affecting lithium levels) were obtained if possible at two or more different timepoints (Study III). Furthermore, lithium response was assessed and reported according to the Alda scala, a 10-point scale summarizing changing in illness activity and possible confounders[188]. Venous blood DNA was obtained at the time of interview. The collection was approved by the Regional Ethical Review Board in Stockholm (#01/389, #04-056).

For study I and II, patients were genotyped using TaqMan® SNP genotyping assays

(cf. 3.2.1). For study III, genotyping was performed in several waves using micro-array platforms (cf. 3.2.3 and Supplementary Material, Study III).

3.1.2 Mayo Clinic bipolar cohort (Study II)

In study II (cf. 4.2), the aforementioned cohort was combined with the bipolar cohort from the Mayo Clinic, based on the Individualized Medicine Biobank for Bipolar Disorder, a collaborative project between the Mayo Clinic, the Lindner Center of HOPE/University of Cincinnati, and the University of Minnesota. This biobank included adults with clinical diagnoses of BD type 1, BD type 2 or schizoaffective disorder that were able to give informed consent. The patients were assessed using the Structured Clinical Interview for the DSM-IV (SCID). Diagnosis, age of onset, comorbidities, demographic variables, clinical variables, and illness characteristics (including lifetime history of rapid cycling) were assessed. Only patients of Caucasian origin were included in the study. Genotyping was performed as described in 3.2.1. The study was approved by the Mayo Clinic Institutional Review Board (IRB# 08-008794).

3.1.3 Swedish depression cohort (Study II)

This cohort of patients with MDD (cf. 4.2) was a subcohort of the PART study, a longitudinal population-based study in Stockholm County, Sweden[189]. Randomly-selected Swedish nationals (N=8613) were asked to respond to an extensive questionnaire on mental health (including the Major Depression Inventory (MDI)), work and relations twice with a 3-year interval. Individuals were considered as having depression when being diagnosed with major depression, mixed anxiety depression or dysthymia, in at least one of the two waves. Diagnosis was set according to DSM-IV, using the MDI. Saliva DNA were obtained from 5527 participants, including all individuals with a depression diagnosis (N = 458). Genotyping was performed as described in 3.2.1. The PART study was approved by the Regional Ethical Review Board in Stockholm (#96-260, #04-528).

3.1.4 MRS cohort (Study II)

Potential participants were referred to the Mayo Clinic and s ened for the presence of a major depressive episode associated with MDD or BD using the SCID. Exclusion criteria included the inability to speak English or provide informed consent, the current treatment with an antidepressant, an history of active substance abuse within the last 6 months, abnormal thyroid-stimulating hormone, unstable medical illness, Young Mania Rating Scale[190] $>$ 12 consistent with hypomania, active suicidal ideation with plan, current psychosis, and antipsychotic treatment within 4 weeks. Overall, 51 individuals were included. For 39 individuals, MRS data acquisition, spectra reconstruction and

quantification were completed. Genotyping was performed for 26 individuals. This study was approved by the Mayo Clinic Institutional Review Board (IRB# 06-006659).

3.1.5 Lithium pharmacokinetics cohort 2 (Study III)

Participants in this cohort were identified using the Swedish National Quality Register for BD (BipoläR) (https://bipolar.registercentrum.se/) and those who consented were included in the SWEBIC-study (Swedish Bipolar Cohort Collection). All patients with BD who receive treatment at psychiatric outpatient clinics in Sweden can be registered and followed in BipoläR. The register contains individualized data on diagnoses, basic clinical epidemiological data, as well as longitudinal data on the natural history and clinical course of the disease, which can be used for research purposes in a de-identified form. For study III, the following variables were obtained: age, sex, body weight, height, creatinine concentration, serum lithium concentration and/or aim serum lithium concentration, when possible, for several timepoints.

This data was then linked with data from the Swedish Prescription registry, where information regarding medication collected from the pharmacies (date, package size, number of packages) is stored. This made it possible to obtain prescription information on lithium and drugs affecting kidney function (antihypertensives (C09), diuretics (CO3) and NSAID). For study III, genotyping was performed in several waves using micro-array platforms (cf. 3.2.3 and Supplementary Material, Study III).

The study was approved by the Regional Ethical Review Board in Stockholm (#2005/554-31/1, #2008/2009-31/2).

3.1.6 Discovery cohort (Study IV)

Twenty-three patients were recruited at the Stockholm Centre for Eating Disorders (SCÄ). This included patients with AN (N = 12) and weight recovered patients with a history of AN (AN-REC, N = 11). In order to be eligible, patients had to be female, 18 years or older, meeting the DSM-IV criteria for AN, with at least 5 years since AN onset. AN-REC patients had to have a history of AN according to DSM-IV and recovered normal weight, defined by BMI > 18, for at least 1 year. Controls (N = 12) were women without own or family history of eating disorders.

Blood was collected at SCÄ and centrifuged within 2 hours, plasma was stored at -80°C and shipped on dry ice. NfL measurements were performed at the Clinical Neurochemistry laboratory at Sahlgrenska University Hospital (cf. 3.4.1).

The study was approved by the Regional Ethical Review Board in Stockholm (#2012/2007-31/1).

3.1.7 ANGI-SE cohort (Study IV & V)

This cohort was a subset from the Swedish sample of the Anorexia Nervosa Genetics Initiative (ANGI-SE), including female patients with active AN (N=113), AN-REC (N=114) and healthy controls (N=113).

ANGI was so-far the largest international research project on the genetics of AN, including biological samples and clinical information from more than 13,000 individuals, many of which were collected at the Karolinska Institutet. These patients were identified through the National Quality Register for Eating Disorders Treatment, the SCÄ and community outreach, and screened using questionnaires, including the ED100K (cf. 3.1.7.1)

For the subcohort, the general inclusion criteria for the AN group were an age of 18 years or more, meeting the DSM-IV criteria for AN (with the exception of amenorrhea) and a minimum illness duration of one year. Individuals in the AN-REC group had to have a history of AN (according to DSM-IV), restored weight (defined by BMI > 20), no eating disorder behaviors (e.g. fasting, purging, excessive physical activity) and normalized eating disorder cognitions. Healthy controls were normal-weight individuals with no record of disordered eating behavior.

Blood was collected at a hospital near the participants, mailed to the Karolinska Institutet Biobank and processed upon arrival. Plasma samples were stored at -80°C and shipped on dry ice. NfL measurements were performed at the Sahlgrenska University Hospital (cf. 3.4.1). Measurements of inflammatory markers were done by Olink Proteomics (Uppsala, Sweden) (cf. 3.4.2).

The study was approved by the Regional Ethical Review Board in Stockholm (#2013/112-31/2).

3.1.7.1 ED100K

ED100K is an online adaptation of the SCID[191], aimed at capturing life-time history on eating disorders[192]. It includes questions on demographics (age, sex, ethnic background, first period), a screening part for eating disorders (height, weight, lowest and highest, eating disorder diagnosis/treatment), and follow up questions on AN. These include questions on weight and height, period of illness, menstruation, weight and body shape perception, eating habits and compensatory behaviors.

3.1.8 Limitations

It is important to remember that results based on clinical material can only be generalized up to a certain point. Indeed, each and every collection of patient material, is biased and can only represent the people actually recruited into a cohort. Clinical cohorts by

definition only represent patients that seek help and are willing to participate in research. Patients not seeking help, as well as those not wanting to participate are therefore not taken into account and the results therefore have to be interpreted with care. Furthermore, understanding which patients decline and the reasons behind their decision will lead to better outcomes, both in research and clinical care[193]. These are limitations, we have to address in our clinical cohorts. While it is easier to address for cohort 3.1.1, which has been well characterized by the clincians and which includes a high percentage of patients treated at the Unit for Affective Disorders in Huddinge, it is more complicated in regards to registry based material. Community outreach and screening, as described in cohort 3.1.7 can be one way to address the problem of non-care seeking patients.

3.2 Genetics

3.2.1 SNP genotyping

For studies I and II, TaqMan® SNP genotyping assays were used. This method is based on polymerase-chain reaction (PCR) using *Taq* polymerase, which has an intrinsic nuclease activity.

For each SNP, two probes were designed. These have exactly the same sequence, except for the interrogated SNP: one probe is complementary to the wild-type allele, while the other is complementary to the minor allele. The probes are linked to a different fluorescent dye each, together with a quencher that suppresses fluorescence emission when the probes are intact. The assays also include a forward primer that guide the polymerase upstream of the SNP. During the PCR, the probes and the primer first hybridize to the targeted sites. The hybridized probes are then lysed by *Taq* polymerase and the fluorescent dyes are released. Depending on zygosity, either one of the dyes (homozygous individual) or both dyes (heterozygous individual) are released. At the end of the assay, the fluorescent signal is measured, and the genotype is determined by software, based on the ratio of the two samples.

3.2.2 Haplotype estimation

Haplotypes are groups of alleles that are inherited together from a single parent. Identifying haplotype associations with diseases has been suggested to increase the power of genetic association studies. Given N SNPs, there are 2^N possible haplotypes. For individuals who are homozygous for all SNPs, the haplotype is unambiguous. For individuals with several heterozygous loci, the haplotype is ambiguous and the only method to resolve this ambiguity is sequencing. It is however possible to estimate the probability of haplotypes in groups of individuals.

In study I, haplotypes were estimated using the *Haplo Stats* library[194]. The estimation is based on a *progressive insertion* algorithm, which combines a expectation-maximization (EM) algorithm, with trimming off pairs of haplotypes with low likelihood. The output includes posterior likelihoods for each haplotype for each individual, as well as haplotype estimates over the whole population and subgroups.

3.2.3 Genome-wide association studies

Genome-wide association studies (GWAS), as their name indicate, are studies that test for associations between a phenotype and genetic variation on a genome-wide level. This analysis allows for unbiased testing that can give rise to new hypotheses. The phenotype can be either binary (i.e. a case/control study), in which case a logistic regression is run for each locus, or a continuous variable, in which case a linear regression model is run. In study III, we performed the analyses using PLINK version 1.90.b.6.9[195], using a linear regression model, with the first four principal-components as co-variates to correct for population structure.

3.2.3.1 Microarry SNP-typing

The genomic data necessary for GWAS is created by SNP-typing: Microarrays that contain 100,000 to millions of small beads, each covered by small probes targeting one specific SNP locus, are incubated with fragmented DNA of the individual to genotype. These DNA fragments will hybridize with their complementary probe. The probes are exactly short one base of the SNP locus and can then be expanded using one of four fluorescent nucleotides. Through laser excitation a color signal dependent on the zygosity (similar to 3.2.1) is generated and can be associated to each SNP positionally. The genotype for each SNP is then determined by software, by identifying clusters of samples with similar intensities[196]. Several quality control steps are then performed: The data is filtered based on minor allele frequency (MAF), low calling rates (i.e. SNPs where many samples have missing information), and samples with low genotyping rate.

Given the structure of the human genome containing regions of high linkage disequilibrium (LD) (haplotype blocks), it is enough to genotype one *index* SNP per region that allows to tag all SNPs that are in high LD. This has led to the fact that common SNP microarrays contain between 500,000 and 1,000,000 variants and has led to the establishment of the generally accepted genome-wide significance threshold of $5 \cdot 10^{-8}$ [197,198]. Based on reference panels produced by projects like the International HapMap Project[199] or the 1000 Genomes Project[200], it is possible to determine untyped genetic variants (tagged by the index SNP), a process termed *imputation*. This is performed using software packages like IMPUTE2[201]. This allows for the interrogation of many more SNPs than those that were directly genotyped.

3.2.3.2 Summary statistics and interpretation of results

For each SNP tested in a GWAS, the results are reported as a table including information on each SNP, odds ratios for binary traits, beta-values for continuous traits, as well as standard error and p-values. These are called *summary statistics* and can be presented as *Manhattan plots*, dot plots showing the approximate position of each SNP on the chromosome on the x-axis and -$\log_{10}$(p-value) on the y-axis.

The significant markers are not necessarily causal, but most of the time highlight a broader genomic region that might contain the functional mutation.[40]. In order to interpret the results several possibilities exist: SNPs of interest can be analyzed individually, in regards to their position (i.e. proximity to surrounding genes) or their potential functional implication (e.g. by testing whether they are expression quantitative trait loci (eQTL), or whether there is 3D chromatin interaction). Furthermore, tools like MAGMA[202] or PANTHER[203] can be used for analysis GWAS results at gene level, as well as gene-set level. Finally, organ-specific transcriptional data, as well as single-cell data has led to the possibility to test whether specific organs or cells have a higher probability to be affected by a set of mutations. In study III, we used FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies)[204], a tool integrating all discussed functionalities, to follow-up our GWAS results.

3.3 Magnetic resonance spectroscopy

Proton magnetic resonance spectroscopy (^{1}H-MRS) is an *in vivo* imaging technique. In a similar way as magnetic resonance imaging (MRI), MRS is based on physical properties of hydrogen protons and can be used to determine the relative quantity of metabolites, including N-acetyl aspartate, creatine, choline, glutamate, myo-inositol, lactate, and GABA[205].

An MR signal is created by applying an external static, homogeneous magnetic field to the area of interest (determined by MRI), and then disturbing it with transversal radiofrequency pulses. Each proton will elicit a signal based on its chemical environment (so called *chemical shift*) that can be detected outside of the body. The key output is an MR spectrum, a graphical representation with the chemical shift for each proton on the x-axis and the peak amplitude, proportional to the amount, on the y-axis. This spectrum is characteristic of each metabolite[205]. Applying MRS to a brain region (usually a voxel of approximately 1 cm^3), the acquisition spectra will contain information for all metabolites, which can be used to calculate metabolite concentrations. Specific MRS protocols have been developed for the quantification of different metabolites[206].

Glutamate and glutamine have a very similar structure, which gives rise to very similar and overlapping spectra. This is why they are often quantified together, a measurement,

which is referred to as Glx[207]. However, with improvements of imaging techniques, it is nowadays possible to estimate glutamate and Glx separately: In study II, a TE-optimized PRESS sequence was used to measure Glx[208], while a two-dimensional J-resolved averaged PRESS sequence was used for quantifying glutamate[209]. Furthermore, measured metabolite concentrations were corrected for CSF volume to obtain tissue volume corrected metabolite concentrations used for statistical analyses[210].

3.4 Protein marker measurements

3.4.1 NfL measurement

Neurofilament light chain (NfL) plasma levels were measured using monoclonal antibodies of an established sensitive sandwich ELISA kit (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden) on the Simoa platform, a bead based immunoassay platform (Quanterix, Lexington, MA, USA). Measurements were performed in duplicates in two rounds (discovery and replication cohort) at the Clinical Neurochemistry laboratory at Sahlgrenska University Hospital. The intra- and interassay coefficients of variation were below 7% for the quality control samples.

3.4.2 OLINK

OLINK bioassays are based on the proximity extension assay (PEA) technology using oligonucleotide labelled antibody probe pairs. When both antibodies bind to the target, the DNA probes are close enough to hybridize. The probes can be extended by a DNA polymerase, which leads to the synthesis of a DNA barcode, which can then be quantified by real-time PCR. This technology allows for the testing of multiple proteins in the same sample (*multiplexing*), as each antibody pair is labelled specifically by the DNA probes[211]. The obtained data is normalized using internal controls and presented as Normalized Protein Expression (NPX) units, a relative quantification unit logarithmically related to protein concentration[212]

According to the manufacturers, the PEA approach offers the same level of performance as ELISA and comparable sensitivity to standard ELISA kits with much less sample (1 μL)[213].

In study V, inflammatory markers were measured using the OLINK inflammatory panel, containing 92 preselected markers, covering a wide range of cytokines involved among others in inflammatory processes. Eighteen proteins were excluded because of high numbers of missing data ($> 20\%$).

3.5 Cell culture

3.5.1 Fibroblasts

Skin punch biopsies were taken from the inner side of the arm of the donors after application of anaesthetic cream. The biopsies were transported to the lab in Dulbecco's modified Eagle Medium (DMEM), supplemented with 20% fetal bovine serum (FBS) and 2% penicillin/streptomycin (P/S). The skin biopsies were cut into small cubes of approximately 0.5 mm^3 and plated onto gelatine coated wells in culture medium consisting of DMEM supplemented with 2% P/S, 20% FBS and 1% non-essential amino acids (NEAA)[214]. The medium was replaced every 2 days. The first keratinocytes could be observed after 3-4 days, the first fibroblasts after approximately a week (Fig. 4.6, A-B). When the cells reached confluence, the cells were dissociated and split (Fig. 4.6, C). Cells were frozen in knock-out serum, supplemented with 10% dimethyl sulfoxide and stored in liquid nitrogen for long-term storage. Fibroblasts were cultured in DMEM, supplemented with 1% P/S, 10% FBS and 1% NEAA.

3.5.2 Induced pluripotent stem cells

iPSC were generated as described in Diecke et al.[215], transfecting the cells using the Thermofisher Neon Transfection System with a codon-optimized mini-intronic plasmid that encodes the canonical reprogramming factors (Oct4, Klf4, Sox2 and c-Myc) together with a short hairpin RNA against p53. One million fibroblasts were washed in Phosphate Buffered Saline without calcium and magnesium (PBS -/-) and resuspended in transfection buffer containing the plasmid. Electroporation was performed according to protocol (1600 V, 10 ms, 3 pulses). The cells were then transferred into full fibroblast culture medium and plated onto laminin-521 coated plates. After three days, half the medium was replaced by *Essential medium 7* (E7, E6 medium supplemented with 100ng/mL FGF2), sodium butyrate (0.1 mM) and ascorbic acid (50 μg/mL). Over the following days, half the medium was replaced on a daily basis. Between day 7-10, the first iPSC were spotted (Fig 4.6, D). The medium was then changed to Nutristem and changed on a daily basis. Once the colonies were big enough, they were manually picked under the microscope, transferred into a single 96-well plate well coated with laminin 521 and expanded (Fig 4.6, E-F). iPSC were maintained on laminin 521 coated plates in Nutristem, changing the medium daily.

3.5.3 Neural stem cells

Neural stem cell (NSC) lines were generated as follows. In a first step, embryoid bodies (EB) were generated by culturing stem cells in suspension on a low-attachment plate in the presence of two SMAD pathway inhibitors to induce neuroectodermal differentiation. Rho

kinase inhibitor was used for the first 24h. After seven days, the EB were transferred to adhesive conditions on poly-L-ornithine (PLO) and laminin coated plates. Thereupon, EB started growing as a monolayer and the formation of neural rosettes became visible after 3-4 days. Neural rosettes were picked up manually and expanded in the presence of epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). NSC were maintained on PLO and laminin coated plates in DMEM/F12, Glutamax, supplemented with N2 (1:100), B27 (1:1000), EGF (10ng/mL) and FGF2 (10ng/mL). B27, EGF and FGF were added every day for the complete volume, cells were split 1:3 upon confluency.

3.6 Statistical analyses

This section covers a selection of the statistical methods used in **studies I-V**, including the replication approaches used in **study I**, linear mixed-effects models (LME), general additive models (GAM) and cross validation methods used in **study III**, as well as quantile regression used in **study V**. The various forms of classical linear regression (t-tests, correlations, ANOVA, multiple linear regression), logistic regression, as well as non-parametric tests (Kruskal-Wallis and Mann–Whitney U test) for p-value based inference are assumed to be well known by the reader and will not be further discussed. All analyses were performed in **R** unless stated otherwise.

3.6.1 Replication

Replication studies are of utmost importance to science, as they provide the standard by which published claims can ultimately be judged. However, there are several ways to interpret results from a replication study[216,217]. You can ask, what is the evidence that a similar effect as originally reported is also present in the replication study? Furthermore, one might wonder how consistent the effect size found in the replication study is with the effect size originally reported? This question is heavily dependent on how one defines consistency (directionality, effect size...)[217]. Finally, one might also ask what conclusions to draw when combining the data. Is there a non-zero effect size that can be detected? How big are these effects? How consistent are the results with one another? However, there is one important question that cannot be easily answered using classical null-hypothesis significance testing (cf. 3.6.3): whether to accept the null hypothesis as true. Failing to reject the null hypothesis is not the same as accepting it[217]. In the following sections, several approaches to solve this problem will be discussed.

3.6.2 Small telescopes

> *Imagine an astronomer claiming to have found a new planet with a telescope. Another astronomer tries to replicate the discovery using a larger telescope and finds nothing. Although this does not prove that the planet does not exist, it does nevertheless contradict the original findings, because planets that are observable with the smaller telescope should also be observable with the larger one.*
>
> – Uri Simonsohn, *Small Telescopes*

One such solution is to test whether the effect is small rather than zero. However, this requires the determination of an effect size that is small enough not to support the hypothesis any longer, which is difficult, as it is inherently subjective. Simonsohn therefore suggested the effect size threshold $d_{33\%}$ based on power of the original study[217]: "*a small effect* [is defined] *as one that would give 33% power to the original study*". Such an effect would have been too small to be of interest to the authors of the original study and can therefore be considered a good threshold. Obtaining an effect size smaller than $d_{33\%}$ in an adequately powered replication is equivalent to saying that the effect size was not large enough to have been detectable by the original sample. To follow up on the telescope metaphor: $d_{33\%}$ corresponds to the size of a planet that is too small to be detected by the small telescope. If a bigger telescope were to detect a smaller planet, it would be considered a failed replication. It is however important to note that the concept relies on the fact that the original study has adequate power for the effect size they are interested in detecting, which is often not the case in biomedical sciences[218].

In study I, we calculated our power to detect $d_{33\%}$ in order to determine whether our study had the size and power to be used as a replication cohort.

3.6.3 Bayesian statistics

In biomedical science, most statistical methods are *frequentist* methods, which are inherently linked to the concept of null-hypothesis significance testing and p-values[219]. Frequentist statistics are based on long-term frequencies of an event occurring or not, i.e. calculating the probability of one outcome over a theoretically infinite amount of repetitions[220]. In frequentist hypothesis testing, obtained data is analyzed in light of a *null hypothesis* of no effect, the p-value being the probability of the data, given that the null-hypothesis is true. The p-value is then compared to a *significance threshold*: if it is smaller, then the data is considered incompatible with the null-hypothesis and the latter is rejected[219].

While frequentist statistics do not attach probabilities to fixed unknown values, but only to the outcomes of repeatable random events, Bayesian statistics define a possible outcome parameter value θ given data D as a probability distribution[219]. A key concept in Bayesian

statistics is the posterior probability distribution $P(\theta|D)$, which is obtained by applying Bayes' theorem:

$$P(\theta|D) = \frac{P(D|\theta) \times P(\theta)}{P(D)} \tag{3.1}$$

In simpler words, the probability of any particular value of θ, given the data D is equal to the likelihood $P(D|\theta)$ times a prior probability $P(D)$, divided by the average likelihood $P(D)$[221].

Inferential testing can be performed using these posterior probability distributions, as intervals in which a parameter lies with a certain probability, can be easily determined[219,221].

3.6.3.1 Bayes factor

The Bayes Factor (BF) is a Bayesian alternative to hypothesis testing. When comparing two hypotheses, e.g. H_1 versus the null-hypothesis H_0 in light of observed data D, the Bayes' factor ($\mathrm{BF}_{10}(D)$) is defined as the ratio of predictive adequacies for the two models. In other words, the ratio between the likelihood of the data D given H_1 and the likelihood of the data D given H_0[222]. When both hypotheses are *a-priori* as likely, this ratio corresponds to the ratio of posterior-probabilities.

BF_{10} is a comparison between two models (note $\mathrm{BF}_{10} = \frac{1}{BF_{01}}$). If $\mathrm{BF}_{10}(D) = 1$, neither of the hypotheses is more likely to have generated D. If $\mathrm{BF}_{10}(D) = 4$, the data D is four times more likely to have occurred under H_1 than under H_0. This characteristic makes the BF very interesting, as it allows to discriminate between absence of evidence ($\mathrm{BF}_{10}(D) \approx 1$) and evidence that would support the null hypothesis ($\mathrm{BF}_{10}(D) \gg 1$)[219].

3.6.3.2 Replication Bayes factor

Based on BF, a so called *replication BF* has been developed as a means to provide a quantification for a replication attempt. It is defined as the "*change in the Bayes factor due to the observation of the replication data and quantifies the additional evidence for the alternative hypothesis given what was already observed in the original study.*"[216]. In other words, the replication BF allows to compare the predictive adequacy of the sceptic's null hypothesis versus an alternative hypothesis that is based on the estimates of the original study and their associated uncertainty. This also means that a replication BF will favor the sceptic's null hypothesis when the effect size of the replication is much smaller than the original reported effect size.

In study I, BF for contingency tables[223] were calculated using JASP[224]. Replication BF were computed according to equation 3.2 as described in Ly et al.[216].

$$\underbrace{BF_{10}(d_{orig}, d_{rep})}_{\text{Complete BF}} = \underbrace{BF_{10}(d_{orig})}_{\text{BF original experiment}} \times \underbrace{BF_{10}(d_{rep}|d_{orig})}_{\text{Replication BF}} \tag{3.2}$$

3.6.4 Linear mixed effects models

One of the assumptions of linear models is the independence of observations. However, data often contains clusters, i.e. datapoints are not independent from each other. When students are sampled from one of several schools, or patients from one of several hospitals, the assumption of independence is violated. The same is true for longitudinal data, where data is sampled at several timepoints from the same individual (cf. 4.3). In these cases, and in particular when there is not the same amount of observations in each group, LME, or multilevel models, can be used to incorporate a hierarchical structure, which leads to better estimates of standard errors corrected for non-independence and allows the analysis of between and within group effects[225,226].

LME incorporate fixed and random effects. Fixed effects are parameters that do not vary, represent the entire population and can be used as explanatory variables. Random effects are usually grouping factors[227] that can be either random intercepts or random slopes. Random coefficients can capture (unmeasured) variability within each cluste that creates variation in the outcome, even when all predictors are similar[221]. These group-level coefficients can be considered to be drawn randomly from a probability model[228], thus the name *random effects* (*fixed effects* being used in contrast). However, many authors agree that the nomenclature is quite confusing[221,228].

In study III, all predictors (e.g. age, height, weight...) were entered as fixed effects, while person-id was entered as a random intercept. These random effects include the inter-individual variability, including the variability due to genetic factors that are not modelled by fixed effects. Their values are also influenced by the amount of observations for each individual.

3.6.5 Generalized additive models

GAM are an extension of generalized linear models, which include a smoothing function. Instead of having a constant relationship (β_1) between an outcome y and a predictor x_1 as shown in equation 3.3,

$$y = \beta_0 + \beta_1 \cdot x_1 + \epsilon,\ \epsilon \sim N(0, \sigma^2) \tag{3.3}$$

this relationship is dependent on a smoothing function $f(x)$, as shown in equation 3.4.

$$y = \beta_0 + f(x_1) + \epsilon,\ \epsilon \sim N(0, \sigma^2) \tag{3.4}$$

This concept is very similar to a relationship that would be described by a quadratic (x^2) or cubic (x^3) function, although the function $f(x)$ is usually more complex[229]. Most commonly, piece-wise polynomials, so called *splines*, are used as smoothing functions. Splines are built up by K simpler base functions that are multiplied by corresponding coefficients and added together to give the final result. K is referred to as base size, the higher it is, the wigglier the spline will be[230].

GAMs were used in study III: GAMs were tested as smoothing functions for estimating daily lithium intake from pharmacy data (cf. 4.3.1). In direct comparison to several other smoothers, a GAM with K=2 gave the best estimates. As can be seen in figure 4.2, the smoothed result is not very wiggly, but is able to capture changes in the amount of lithium taken, while not appearing to overfit the data. Increasing to K=3 made the smoother more sensitive to outliers, which yielded worse prediction results. Splines were also used to model the non-linear effects of age and eGFR on the outcome variable.

3.6.6 Cross-validation

Cross-validation (CV) refers to model validation to check how results from one statistical model will generalize to an independent data set. CV is an important technique for detecting overfitting, a common problem in statistical modelling, and is particularly important in situation where models are not only used for *inferential* but also used for *prediction* purposes. CV involves the separation of a sample into two complementary (i.e. non-overlapping) subsets, building a model M in one subset (called the *training set*) and testing it in the other (*testing set*). Testing consists of predicting the outcome variable based on the predictor variables and the coefficients of the model M. Most of the time, several rounds of CV are performed, in order to reduce variability[221].

Several cross-validation models exist, here leave-one-out (LOO) CV, as well as out-of-sample (OOS) CV used in study III will be discussed. In the LOO CV, a sample with N observations, is split into a training set of $N-1$ samples and tested on the sample that was left out. This procedure is repeated N times, after which measures of fit are calculated by comparing the measured values to the predicted values. In the OOS CV, a completely different sample that contains the same information as the original sample used to build the model is used as testing set. This procedure is only performed once and the measures of fit are calculated[221].

Measures of fit depend on the structure of the data and the model. In study III, we report Root-mean-square error (RMSE) and the Pearson correlation coefficient, as measures of fit. The RMSE corresponds to the square root of the average of squared errors.

3.6.7 Quantile regression

Linear models based on ordinary least squares (OLS) methods are used to model the relationship between the **mean** of the dependent variable and levels of independent variables. Several assumptions have to be met in order for the model to work. Alongside the assumption of independence (cf. 3.6.4), linear models require the residuals to be normally distributed. Furthermore, when the data is very skewed, or includes many outliers, modelling the mean might not be greatly informative[231,232].

Quantile regression is a regression model that models the relationship between the median (or any other quantile) and the dependent variables. The model does not rely on any assumption on residual distribution, is more robust against outliers and allows to explore relationship in the data that are not represented by the mean, allowing for example to explore how predictor variables affect the extremes of a distribution[231,233]. While OLS methods minimize the sum of the squared errors, median regression minimizes the sum of absolute errors. While OLS produce an unique solution, quantile regression can have more than one single solution[233].

In study V, 27 markers could not be modelled by linear regression, as the distribution of the residuals would have been highly skewed. We therefore decided that for those markers, modelling the median was more informative than modelling the mean. Modelling was performed using the quantreg package[234], p-values were derived by bootstrapping[232].

Chapter 4

Results and Discussion

The studies included in this thesis cover genetic and biomarker analyses on bipolar disorder and anorexia nervosa. While this section includes a summary of the results, a more extensive discussion of each study can be found in the respective paper or manuscript. In a final section, I also shortly summarize non-published data on the establishment of *in vitro* models and discuss their potential use in translational psychiatry.

4.1 Study I

The serine/threonine kinase AKT is a central player in intracellular signal transduction, with a role in many physiological and pathological settings. It is situated downstream of the phosphoinositide 3-kinase (PI3K): Activation of receptor tyrosine kinases or G-coupled receptors leads to the activation of PI3K and the production of lipid products (e.g. phosphatidylinositol (3,4,5)-trisphosphate (PIP_3)). These recruit inactive AKT to the cell membrane, where it is phosphorylated and thereby activated. The signalling process is terminated by lipid phosphatases acting on the lipid products, as well as phosphatases that directly dephosphorylate AKT. Hundreds of substrates have so far been reported, one of the best studied ones is GSK-3β[235].

The AKT/GSK3β pathway is well studied in psychiatry. In particular *AKT1* is thought to be involved in the pathogenesis of schizophrenia and psychosis, which is supported by early genetic studies, *post-mortem* studies, animal experiments, as well as pharmacological studies[236]. Indeed, many antipsychotic medications have an influence on AKT activity by modulating dopamine signalling[237]. Finally, lithium, a first line therapy for BD, has a well-known impact on the AKT/GSK3β signalling pathway, both through a direct inhibitory effect on GSK3β, but also influencing AKT phosphorylation[80].

AKT1 has also been suggested as a risk gene for BD and was originally brought forward as candidate gene for BD by Toyota et al.[238]. Karege et al.[239] reported several haplotypes in

AKT1 to be associated with varying occurrence of BD: While the **TC** haplotype, formed by the SNPs rs1130214 and rs3730358, was associated with a lower risk for BD, the **GC** haplotype formed by the same SNPs supposedly increased this risk. Two years later, the same group described associations between several SNPs and subphenotypes of BD (based on history of psychotic episodes)[240].

4.1.1 Results

As these studies were based on relatively small samples, we aimed at replicating these results in a much larger cohort of BD patient (cf. 3.1.1), with information on the same subphenotypes.

We calculated the power for three thresholds: (1) for the reported main effect sizes, (2) for an effect size equal to half of the reported size, (3) for an effect size, which the original studies had 33% power to detect ($d_{33\%}$). This last threshold is based on the concept *small telescopes* brought forward by Simonsohn[217] (cf. 3.6.2). The idea behind it is that an effect size that could only be found with 33% power would not be of interest for the original authors and has been suggested as being a possibility of answering the question whether the replication study suggests that the effect of interest is undetectably different from zero.

Our study had 99% power to replicate the main effects, above 94% for effect size equal to half the reported size and above 77% for $d_{33\%}$ and we therefore concluded that it was adequately powered for replication.

None of the results reported to be statistically significant in the original study were statistically significant in our replication study.

In order to strengthen these findings, we performed a Bayesian analysis, calculating Bayes Factors (BF) for all comparisons, as well as replication BF (cf. 3.6.3). The BF allows for a quantification of the support of one model over the other, while the replication BF gives an information on how much support there is in the new data for a successful replication of the original data, relative to a failed replication. The BF using default (i.e. flat) priors supported the null hypothesis in our new data, with moderate to strong evidence. The replication BF showed very strong evidence against the originally reported effects, favouring the null hypothesis by a factor between 5 and 1327 times.

In a final step, we studied the results of the latest GWAS for schizophrenia[241] and BD[44]. No SNP in a 20kb range of the *AKT1* gene showed an association with any of these disorders.

4.1.2 Summary

In this study, we were unable to replicate previous results on genetic associations between BD and genetic variation in the *AKT1* gene[239,240], using frequentist, as well as Bayesian approaches, with support from publicly available GWAS results.

4.1.3 Limitations

On top of limitation addressed in our publication, which include the retrospective assessment of phenotypes, as well as a lack of information on healthy controls, it is important to mention that we are fully aware of the fact that candidate approaches are nowadays outdated. However, as so far GWAS on subphenotypes are scarce, the specific subphenotypes reported in the original studies have not been studied at all using GWAS and the original articles are still being cited, we decided it was important to publish this data.

4.1.4 Personal comments

This study was one of the first studies performed during this PhD thesis and was meant to build up onto a larger study on the role of AKT/GSKβ signalling, especially in light of lithium response. However, as the first step, the replication of the studies by Karege et al. was unsuccessful, and upcoming GWAS studies on BD did not give any indications on an involvement of *AKT1* in BD, we decided to stop the project. The publication process of this data proved to be difficult. This study exemplifies all projects with negative results, published or unpublished, that I have encountered during my doctoral studies.

4.2 Study II

Glutamate is the most abundant excitatory neurotransmitter in the CNS and is involved in multiple important functions, including synaptic plasticity, memory and learning. The regulation of glutamate levels in the brain is therefore of utmost importance, especially as excessive extracellular glutamate levels are considered neurotoxic. This *glutamate excitotoxicity* has been associated to several acute (e.g. epilepsy, ischemia), and chronic brain disorders (e.g. amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease)[242]. Furthermore, dysregulation of glutamate neurotransmission is thought to play a role in the pathophysiology of a number of psychiatric disorders, including schizophrenia, BD and major depressive disorder[206]. Perhaps the most striking example of a role for glutamate in affective disorders are the rapid anti-depressant effects of the *N*-methyl-D-aspartate (NMDA)-receptor blocker ketamine.

The implication of dysregulated glutamate in the pathophysiology of BD is supported by several lines of evidence. While *post-mortem* studies have shown reduced expression of both ionotropic and metabotropic glutamate receptor subunits in several brain regions[243–246], recent genetic studies have associated several genes involved in glutamatergic signalling

with BD, for example the NMDA-receptor subunit GRIN2A[44] or the *SYNE1/CPG2* gene[247]. Further support comes from imaging studies, in particular ^{1}H-MRS studies (cf. 3.3). As discussed in section 1.2.3.2, BD has been associated with elevated Glx and glutamate levels in ACC and prefrontal regions[53,54]. In contrast to these findings, meta-analyses on major depressive disorder found lower glutamate and glutamine levels in ACC[248], and the PFC[249].

Few studies so far have analyzed the effects of genetics on glutamate levels in the brain, mostly in the context of schizophrenia spectrum disorders[250,251]. A recent twin study analyzed the heritability of several metabolite levels in the thalamus and the ACC in the context of schizophrenia spectrum disorders. The authors report heritability estimates of about 0.3 for both glutamate and Glx in the ACC[252]. In bipolar disorder, a genetic variation in bcl-2 was associated with changed Glx/Creatine in ACC[253], while the val66met polymorphism in the *BDNF* gene was associated with changes of Glx/Creatine in the left hippocampus[254].

4.2.1 Results

In this study, we aimed at exploring the effects of genes of the glutamate circle on Glx and glutamate levels in the ACC and the left dorsolateral PFC in a mixed cohort of depressed patients. We included SNPs in essential regulatory elements and coding sequences of *GLUL*, *SLC1A3*, *SLC1A2*, and *SLC1A7* encoding for glutamine synthetase, excitatory amino acid transporter (EAAT) 1, EAAT2 and EAAT5, respectively.

While no difference was found between patient with MDD or BD in any of the regions, the minor alleles of rs3812778 and rs3829280, two SNPs in perfect linkage disequilibrium (LD) in the 3'-untranslated region (UTR) of *SLC1A2*, were associated with elevated 2D JPRESS mean ACC glutamate levels. No associations were observed for the combined glutamate/glutamine levels.

In silico analysis using the BrainCloud eQTL browser revealed an association between the minor allele of rs3829280 and higher levels of *CD44*, a gene situated downstream of *SLC1A2* on chromosome 1. These results were corrobated by data from the UK Brain Expression Consortium, where associations in the same directions were found between the full-length transcript and rs3812778 in the cerebellar cortex, the putamen, the substantia nigra, as well as in the average of all brain regions.

CD44 codes for a receptor for hyaluron and plays important roles in cell-matrix binding, signaling, cell migration[255], inflammation[256] and has been shown to play an important role in brain development[257]. Having also been identified as a marker for astrocyte precursor cells[258], we assessed the correlation with several astrocytic markers and markers of the glutamate-glutamine cycle and found very high correlation of *CD44* with markers expressed in astrocytes (*AQP4*, *GFAP*, *S100B*, *GLUL*, *SLC1A3*, *SLC1A2*, *SLC38A3*), but not with

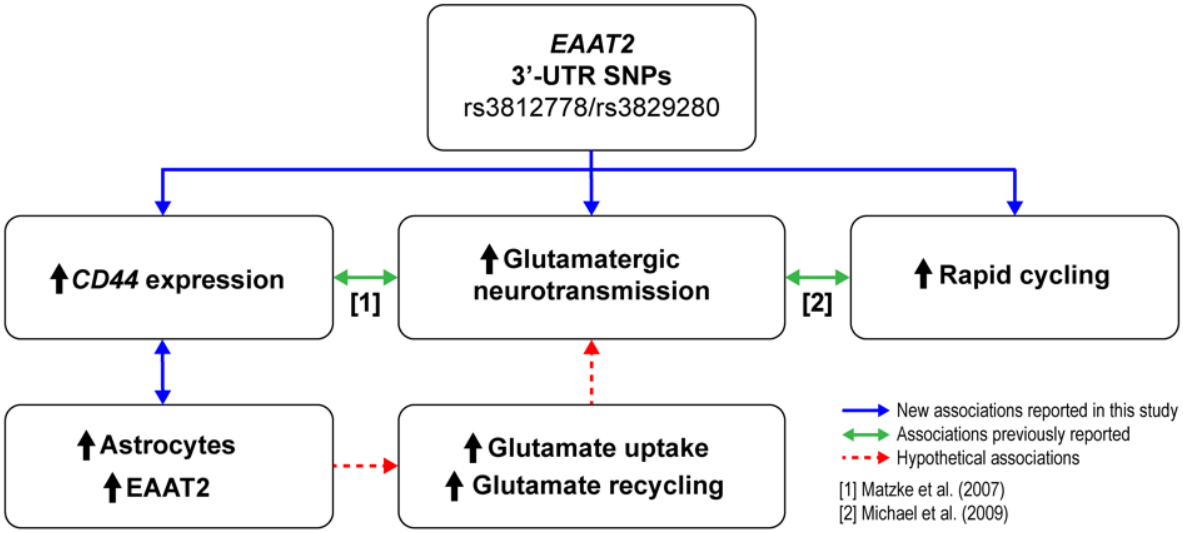

Figure 4.1: Pproposed Mechanism for the Impact of Genetic Variations in *SLC1A2* on Glutamatergic Neurotransmission and Rapid Cycling. Figure from Veldic, Millischer et al., 2019, *Translational Psychiatry*.

those expressed in neurons (*GLS*, *SLC1A1*, *SLC1A6*, *SLC38A1*, *SLC17A7*, *SLC17A6*, *SLC17A8*, and *SLC1A7*).

Finally, to explore potential disease relevance, we followed up the finding in a mixed cohort composed of both MDD and BD. While no significant difference in the percentage of minor allele carriers was found between patients with MDD and patients with BD, patients with rapid cycling BD had a significantly higher percentage of minor allele carriers (26.9% [95% Confidence Interval (CI): 23.5–30.5]) in comparison to non-rapid cycling (21.8% [95% CI: 17.9–25.8]) or patients with MDD (21.9% [95% CI: 19.1–24.9]).

4.2.2 Summary

Based on our findings, we propose a mechanism presented in Fig. 4.1. We hypothesize that the minor alleles of rs3812778/rs3829280 are associated with increased levels of *CD44*, possibly a sign of higher numbers of astrocytes. This increase of EAAT-expressing cells could lead to increased glutamate recycling, dysregulation of glutamatergic neurotransmission, and increased risk for rapid-cycling. While some of these association have newly been reported by this study (*blue arrows*), other associations in this hypothesis (*green arrows*) have been reported previously[259,260].

4.2.3 Limitations

There are several limitations to our study. The main one being the small number of patients included in the MRS study. Furthermore, not all genes that could have an impact on the glutamate levels were analysed. A replication in a bigger cohort, including a more complete list of genes (and their genetic variation) is therefore necessary. In order to address the power problematic, such a study would most probably have to be performed in a collaborative manner.

Furthermore, ^{1}H-MRS measurements of glutamate have an intrinsic limitation, as this method captures the total concentration of glutamate in the area of interest, making it difficult to separate (1) intra- and extracellular glutamate, as well as (2) glutamate used for metabolic functions from glutamate used as neurotransmitter[53].

Finally, there is some heterogeneity in the diagnoses of BD and MDD. While BD diagnosis was based on medical interviews, medical records and questionnaires performed in clinical settings, MDD cases where selected from a population cohort using the Major Depression Inventory.

4.3 Study III

Pharmacokinetics is the study of the fate of pharmacological substances in the body, describing their absorption, distribution, metabolism and excretion. Traditional pharmacokinetic studies are usually based on repeated measurements from the same individual at fixed time-intervals after a given dose. These studies often only include few patients and are used to calculate pharmacokinetic parameters such as bioavailability or clearance. In contrast to this, population pharmacokinetic studies are based on measurements from patients treated with the drug. Although they require much larger cohorts, fewer measurements per patient are needed. Furthermore, this study design allows one to distinguish between inter- and intra-individual variability and makes the analysis of co-variants (e.g. age, sex, kidney or liver function) possible[261,262].

In this study, we used a population pharmacokinetic approach to investigate lithium pharmacokinetics, using the log-ratio between serum lithium (SL) and daily lithium intake (DLI) as outcome of interest. This ratio corresponds to the inverse of log(lithium total body clearance) at steady state, assuming a bioavailability of 100%.

Lithium pharmacokinetics is a well-studied field, both at individual and population level. However, most of the studies were based on small sample sizes and genetic effects have so far not been studied. In order to achieve enough power to perform a GWAS (cf. 3.2.3), we therefore based our study on two Swedish cohorts (cf. 3.1.1 and 3.1.5), including overall 5759 observations from 2452 bipolar patients treated with lithium.

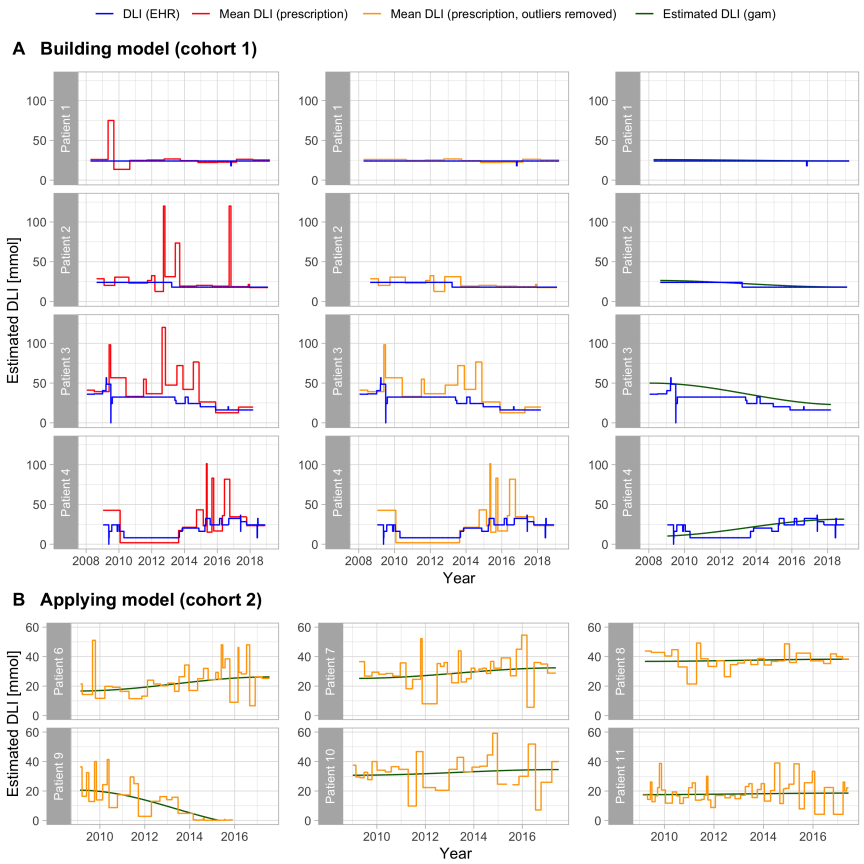

Figure 4.2: Prediction model to estimate daily lithium intake based on amount of lithium collected at pharmacies. **A Building the model.** Column 1: Examples of the relationship between daily lithium intake (DLI) from the electronic health records (EHR) and the mean DLI per prescription. Column 2: Relationship between DLI (EHR) and DLI (prescription) after outlier removal. Colum 3: Relationship between DLI (EHR) and estimated DLI using a generalised additive model (k=2). **B Applying the model**. Exemplified application of the model on the data of cohort 2 after outlier removal. Figure from Millischer et al., *manuscript*.

4.3.1 Estimation of daily lithium intake from prescription data

In cohort 1, we had access to the patient's electronic health records (EHR) and therefore to the information on the number of tablets prescribed. In cohort 2, based on register data, we only had access to the information of the Swedish prescription registry and therefore to the amount of medication collected at the pharmacies (date of purchase, size and number of packages collected). In a first step, we therefore built a prediction model to estimate daily lithium intake based on this data in cohort 1 (exemplified in Fig. 4.2).

After collecting both type of information from 87 patients from cohort 1, we calculated the mean daily lithium intake per prescription period (mean $\text{DLI}_{\text{prescription}}$) by dividing the total amount of lithium prescribed by the time until the next prescription (Eq. 4.1).

$$meanDLI_{prescription} = \frac{n_{tablets}.mol_{tablet}}{time_{prescription}} \tag{4.1}$$

Column 1 in Fig. 4.2-A shows the relationship between this estimate (red line) and the DLI recorded in the EHR ('DLI (EHR)', *blue line*). After removing outliers (column 2, *orange line*), we tried several smoothing models (median, mean, LME, GAM) in a training set (N=60) and tested the best two in a testing set (N=27). The best fitting model was the GAM with k=2 (column 3, *green line*), with a RMSE of 6.716, which corresponds to an error of approximately one tablet of lithium sulfate. This model was then applied on all patients in cohort 2 (Fig. 4.2-B). An estimated DLI value was thus obtained for all timepoints in the quality registry between the first and the last lithium prescription.

4.3.2 Influence of demographic, physiological and clinical parameters on log(SL/DLI)

We first studied the effects of demographic, physiological and clinical parameters on log(SL/DLI), which allowed us to replicate several well-established findings. In univariate analysis using a LME (cf. 3.6.4) with individuals as random effects, we could show associations between log(SL/DLI) and age, sex, body weight, height, estimated glomerular filtration rate (eGFR), serum lithium level, co-medication with diuretics (excl. potassium sparing agents) and agents acting on the renin-angiotensin-aldosterone system (RAAS). The effects were very similar in both cohorts, providing evidence that the estimation of DLI in cohort 2 was correct.

These results indicate that older patients, women, patients with reduced kidney function, lower body mass, as well as patients taking diuretics or agents acting on the RAAS require lower doses of lithium. Furthermore, the positive association between SL and log(SL/DLI) is an indicator that increases in DLI are not linearly associated with increases in SL. In other words, in order to increase SL from 0.4 to 0.5 mmol/L a higher increase in DLI will be needed than to increase SL from 0.8 to 0.9 mmol/L. This effect was not only observed at the sample level, but also within individuals.

A LME including all predictors as fixed effect and individuals as random effects explained 63% and 47% of the variance of log(SL/DLI) in cohort 1 and cohort 2, respectively, with marginal effects being very similar between both cohorts. Cross-validation in a leave-one individual out (LOO), as well as an out-of-sample (OOS) fashion (cf. 3.6.6) showed good fits for both models.

4.3.3 The influence of genetic factors on log(SL/DLI)

In the next step, the random effects of the previous model were used as the dependant variable in a GWAS. These random effects represent the individual-level effects in the SL/DLI ratio after accounting for the above-mentioned co-variates. In other words, they represent the inter-individual differences that are not explained by any of the predicting variables. These are – at least partly – due to genetics. A GWAS was run in both cohorts separately, including the four first principal components as covariates to control for population substructure, followed by a meta-analysis.

No locus was found to be significant at genome-wide significance level, in either of the cohorts separately or in the meta-analysis. Nine genomic loci showed an association at $p < 10^{-6}$ in the meta-analysis; the strongest one was rs61761173 on chromosome 1 in an intronic region of *MEGF6* ($p=1.10^{-7}$).

A gene-based analysis using MAGMA revealed the gene *QSER1* to be significant after correction for multiple testing ($p_{adj}=0.036$). Although its function is currently unknown, *QSER1* was found to be associated with diabetes and obesity in several studies[263–265]. Interestingly, the third strongest association was for *FTO*, a gene that has also been associated with several metabolic traits (e.g. type II diabetes[266], body fat percentage[267]). These results would support the hypothesis that genes affecting body composition have an impact on log(SL/DLI). Finally, gene ontology analysis of 27 genes that were prioritized using FUMA[204] showed an enrichment for genes involved in metal homeostasis. This result was mainly driven by a number of genes in the metallothionein (MT) family. Present in all tissues, MT are able to bind transition metal ions with high affinity, and are involved in the response to zinc, copper, and cadmium ions. Even though, so far, binding to lithium has not been shown, an implication of MT in lithium metabolism seems plausible.

4.3.4 Prediction algorithm

Based on these results, we developed an algorithm to predict the amount of lithium needed to reach therapeutic levels, including the eight parameters described in section 4.3.2. As no single genetic variable with a big effect size was found to influence log(SL/DLI), we decided not to include genetics into the prediction model. Indeed, even though the inclusion of a polygenic risk score (PRS) based on our results might improve the model, performing a full genotyping of patients before starting lithium therapy seemed clinically unfeasible.

The algorithm has been implemented as an interactive *shiny* app[268], currently available upon request and requiring *R-studio* to run. It requires the input of sex, age, weight, height, plasma creatinine, co-medication, as well as the required serum lithium concentration and the lithium preparation. The output comprises (1) a summary of the input including the eGFR from creatinine input (*top table*), (2) a table giving the approximate amount of tablets needed to reach different serum concentrations, as well as the 95% prediction

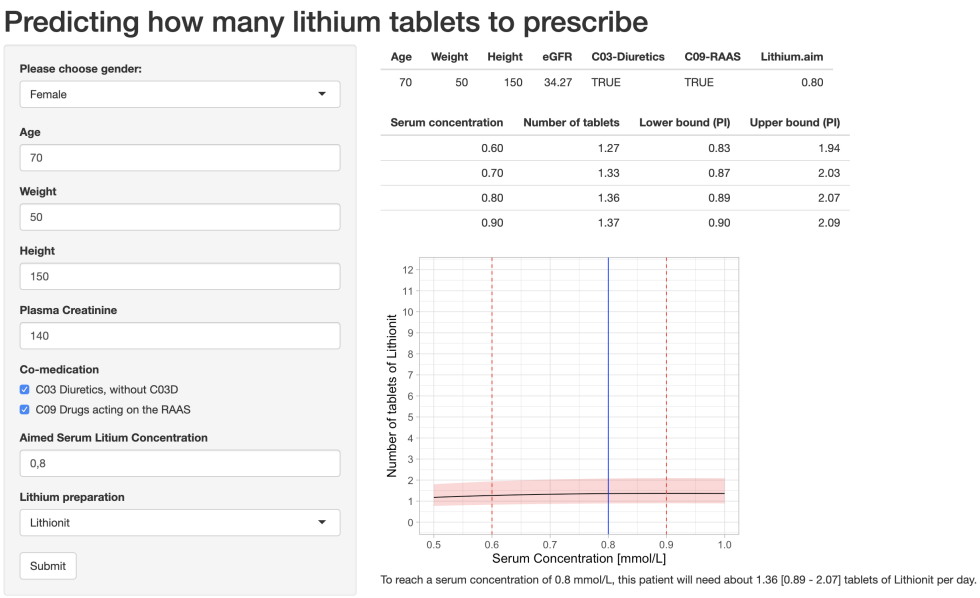

Figure 4.3: Using the prediction algorithm to predict the amount of lithium needed for an elderly woman with chronic kidney disease.

interval (PI) (*second table*), (3) a graphical representation of the same information, and finally (4) a written sentence as summary.

Two examples are shown here in fig. 4.3 and 4.4. While fig. 4.3 shows the prediction for an elderly woman with chronic kidney disease (0.89-2.07), fig. 4.4 shows the prediction for a young, healthy but overweight man (4.05-9.24). As expected, given the amount of variance explained by the model, the prediction interval is relatively large. Furthermore, it is worth noticing that the prediction line shown in the graphical representation is fairly flat. This effect is due to the non-constant relationship between log(SL/DLI) and SL (cf. 4.3.2) in our model, which predicts a sharp increase of SL above a certain threshold of DLI. The mean error in predicting DLI estimated from the LOO cross validation is about 6 mmol. Although these errors show that our model is too uncertain to blindly rely on the algorithm, we think that it could provide a useful guideline for clinicians, by summarising data that is collected before starting a lithium therapy in an evidence-based matter. Having a theoretical target value for the amount of lithium needed from the beginning could eventually lead to a shorter time needed to reach therapeutic levels, as well as reduced adverse events due to too high SL levels. The usefulness of the application has yet to be determined in a prospective study.

4.3.5 Concluding remarks and future plans

This is the first study combining classical pharmacokinetic approaches with a GWAS to study lithium pharmacokinetics. Although no single locus was found to be significant, the

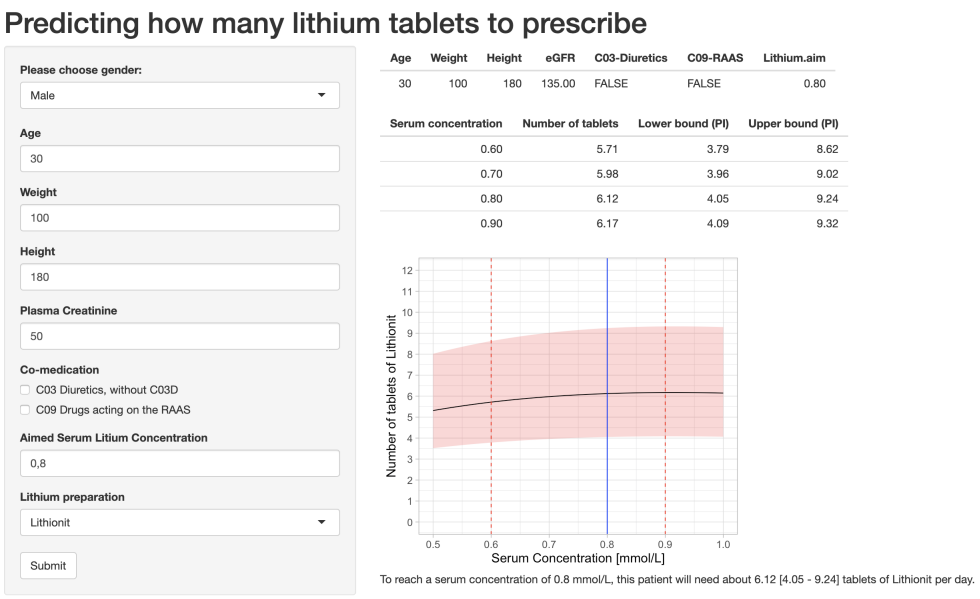

Figure 4.4: Using the prediction algorithm to predict the amount of lithium needed for a young healthy man.

analysis of the GWAS results uncovered several interesting findings, not least that there is no single genetic variation with a large effect size influencing log(SL/DLI). Furthermore, we were able to confirm several known findings in the biggest pharmacokinetic study on lithium so far, and use these results to build a prediction algorithm. In order to strengthen these results and bring them closer to clinical application, we are planning several follow-up projects.

The prediction algorithm will be validated in two phases. In a first phase, we have started to collect data from lithium naïve patients during lithium therapy initiation until steady-state is reached. The analysis of this data will allow us to determine whether the algorithm works in a prospective way, i.e. whether the variables before treatment have the same explanatory power for log(SL/DLI), as when collected simultaneously. In a second phase, we will perform a prospective study: We will compare the time needed to reach therapeutic levels and the number of adverse events during lithium therapy initiation with and without the use of the prediction algorithm. If the efficiency of the tool can be validated, it would be easy to implement in clinics, providing a method to a personalizing treatment in psychiatry.

Regarding the genetic part of the study, we are planning to first cross-validate the genetic results using a PRS calculated from each cohort independently in the other cohort to see how strong the explanatory power would be. In a second step, we will try to increase our sample size by contacting our collaborators in the ConLiGen. Given our results, we are positive that increasing the sample size will lead to the discovery of genome-wide significant results that could then eventually be used to improve the prediction algorithm.

4.3.6 Limitations

There are several limitations to the study. The estimation of DLI in cohort 2 introduces some variation, however, as the results for both cohorts were very similar, we are confident in our results, even though some variation remains unaccounted for. Also, some variables that probably influence our outcome of interest are missing: NSAID intake, morning/evening intake of lithium, as well as the interval between the last lithium intake and blood draw. The use of NSAID is not possible to correct for when being bought over the counter. However, lithium treated patients are strongly advised against using NSAID without prescription and prescribed NSAID did not appear to influence log(SL/DLI). Furthermore, we only had information about the total daily amount of lithium and not how many tablets are taken mornings and evenings. This might influence the lithium levels the next morning: for the same number of tablets daily, a patient taking more in the evening might have slightly higher levels in the morning than a patient that takes more in the morning. Finally, patients are advised to draw blood exactly 12 hours after the last lithium intake, however, this is not always possible. We estimate however that given the known half-life, a couple of hours will not significantly influence SL levels.

4.4 Study IV

Neurodegeneration is a process present in many neurological disorders, both in classic neurodegenerative disorders, e.g. Alzheimer's disorder[269], amyotrophic lateral sclerosis[270], Parkinson's disorder[271], but also immune mediated disorders like multiple sclerosis[272]. Neurodegenerative processes have also been suggested to play a role in psychiatric disorders, like BD[31]. As described in section 1.3.3.3, volume reductions in several brain areas affecting both grey and white matters have been described in patients with AN and point toward possible neurodegenerative processes associated with AN.

Neurofilaments are intermediate filaments exclusively located in the cytoplasm of neurons, which provide structural stability to neurons. They are formed by three types of subunits, neurofilament heavy chain, medium chain and light chain (NfL)[273]. NfL is the most common and most soluble of the subunits, which makes it easily measurable in both CSF and peripheral fluids and therefore a good candidate biomarker for neurodegenerative processes[273]. Indeed, even though NfL is constantly released into the CSF at low levels in an age-dependant manner[274], this release increases sharply during inflammatory or neurodegenerative processes[275], as well as during traumatic or vascular brain injury[274]. Based on these properties, NfL has been extensively studied and associations with several neurodegenerative disorders have been established, among others with multiple sclerosis[276], Alzheimer's Disorder[277], frontotemporal dementia (FTD)[278], traumatic brain injury[279]. Interestingly, NfL has been brought forward as a potential biomarker to distinguish FTD from AD, as well as from psychiatric disorders that would mimic FTD symptoms[273]. As

for psychiatric disorders, elevated NfL levels have been reported in the CSF of patients with BD[280].

4.4.1 Results

We measured NfL in the plasma of patients with AN, patients that had recovered from AN (AN-REC), as well as healthy controls in two different cohorts in an attempt to better delineate potential neurodegenerative processes in AN.

After replicating the previously published association between NfL levels and age, we report higher levels of NfL in plasma of patients with AN compared to healthy controls and to AN-REC in both cohorts. Higher levels of NfL in AN-REC compared with controls were observed only in the larger replication cohort. Furthermore, we found a negative association between NfL levels and BMI across all samples. Interestingly, the slopes of the association were significantly different in the AN group compared to both AN-REC and controls: NfL levels increase sharply with lower BMI below a BMI of 18.5. This is an indication that the increases in NfL levels observed in AN are not only a linear effect of lower BMI, but that separate or accelerated processes appear below a certain threshold.

Overall, our results show that elevated levels in active AN might normalize somewhat with weight recovery. The 2-fold increase observed in AN patients is however lower than the observed changes in MS[281] and corresponds roughly to the increase observed in mild traumatic brain injury in boxers[282]. It has however to be noted that comparisons across studies have to be performed with care.

The origin of this increase can only be object of speculation. Even if serum and plasma concentrations of NfL are not influenced by blood-brain-barrier integrity[283], correlate highly with concentrations in the CSF[277,281,283], increased peripheral NfL levels could also come from peripheral nerve damage. The central origin of NfL seems nonetheless an appealing hypothesis, and would be supported by imaging studies, as well as the neurodegeneration seen in the hypothalamus of the spontaneously anorectic *anx/anx* mouse (cf. 1.3.3.7).

There are, to our knowledge, two previous studies that analysed possible markers of neuronal damage, including S100B[284], glial fibrillary acidic protein (GFAP) and neuron-specific enolase[285]. However, no changes in the levels of these markers was observed between patients with AN and controls. This could be due to smaller sample size, or to different mechanisms leading to the increase of these markers. Indeed, S100B was not elevated in traumatic brain injury[279] or BD[280], while NfL was.

4.4.2 Limitations

There are several limitations in our study. Although our study supports the hypothesis that neurodegenerative processes occur during the acute phase of AN, the lack of synchronized

imaging on the same individuals makes it impossible to determine where these processes are actually occurring. A parallel measurement of NfL in CSF would also have been an advantage. Furthermore, our study was cross-sectional, which makes conclusions on the development over time more difficult. This is especially true for the recovered group: Even though a normalization after weight gain seems plausible, we were not able to conclude anything on the time course of this effect, as the information on length of recovery was not available. Finally, as for many other studies on AN, it is impossible to determine whether the observed effect is due to the low BMI or an AN-specific process. Including constitutionally lean individuals might help clarifying this problem.

4.4.3 Concluding remarks and future plans

This is the first report on elevated NfL levels in AN compared to healthy controls, as well as AN-REC, pointing toward neurodegenerative process in the active illness, which might be attenuated upon recovery.

In a next step, it will be interesting to analyse NfL levels in a longitudinal manner, in order to define the development over time after recovery and assess whether increased NfL levels can be used as predictors for recovery. It would be of particular interest to measure NfL levels, both centrally and peripherally, in patients undergoing imaging analyses in order to determine the origin of the increased NfL levels.

4.5 Study V

Building on the potential role of inflammation (cf. 1.3.3.5) and markers seen in anx/anx mice, we decided to further investigate the role of inflammation in AN. Using the *inflammation panel* from OLINK (cf. 3.4.2), we measured a broad array of inflammatory markers in the plasma of a large sample of persons with AN, AN-REC and healthy controls. After quality control, we included 74 markers in our analysis.

For each one of these markers, we assessed group differences (i.e. comparing AN, AN-REC and controls), as well as associations with BMI. In order to assess whether the associations with BMI were linear (in a similar way as described in section 4.4), as well as to make a comparison with earlier studies that do not include patients with low BMI, we analysed the BMI over the whole range, as well as in the AN-group (BMI < 18.5) and the non-AN group separately (BMI > 18.5). Many of the marker levels were not normally distributed. Being log-transformed, we opted for using quantile regression (cf. 3.6.7) in order to model the median instead of the mean for those markers.

After Bonferroni correction, we report 19 proteins to be significantly lower in AN compared to controls, most of which were also significantly lower between AN and AN-REC. Six proteins had significantly higher plasma concentrations in AN compared with controls, three of which were also significant between AN and AN-REC. Not a single marker was significantly different between AN-REC and controls (Fig. 4.5 A).

Furthermore, we report twenty-five proteins with a positive correlation with BMI and four proteins with a negative correlation over all samples (Fig. 4.5 B). Interestingly, while most of the markers with a positive correlation (column B, blue) over all samples also showed some correlation with BMI in the subgroup of patients with BMI > 18.5, none of the markers with a negative correlation over all samples (column B, orange) appeared to be correlated with BMI in the said subgroup.

It is important to note that many of the *inflammatory* cytokines also have other roles not directly linked to inflammation. This is well exemplified with a subgroup of proteins involved in bone physiology that we have found to be different between AN and controls (OPG, TRANCE (RANKL), CSF1 (MCSF) LAP-TGFβ, and CST5). Low bone mineral density and increased risk of fractures is a common complication of AN[163]. This could be caused by decreased estrogen concentrations, low BMI, elevated cortisol levels, low IGF1 levels, as well as poor nutrition and a lack of vitamin D[163]. Some evidence indicates that body weight restoration can reverse bone loss, which is supported by our findings[163]. While being indicative of a disturbed balance between bone formation and resorption in acute AN, none of these proteins differed between AN-REC and controls, suggesting that these effects are reversed upon weight gain/restoration even though although longitudinal studies are needed for confirmation.

4.5.1 Limitations

There are similar limitations in this study as in study 4. Despite being one of the first studies to include an age-matched group with women who had recovered from AN, the cross-sectional design of our study cannot establish cause and effect. Also, the differentiation between state (low BMI) and trait (AN) is difficult without constitutionally lean individuals, even if the result of our study would support the state hypothesis for most markers—as no differences between AN-REC and controls were seen. A longitudinal study following persons with AN during the course of recovery is necessary to address both of these problems.

4.5.2 Concluding remarks and future plans

This is, to our knowledge, the largest study on inflammatory markers in AN, both regarding sample size and number of markers studied. We report an aberrant inflammatory profile in

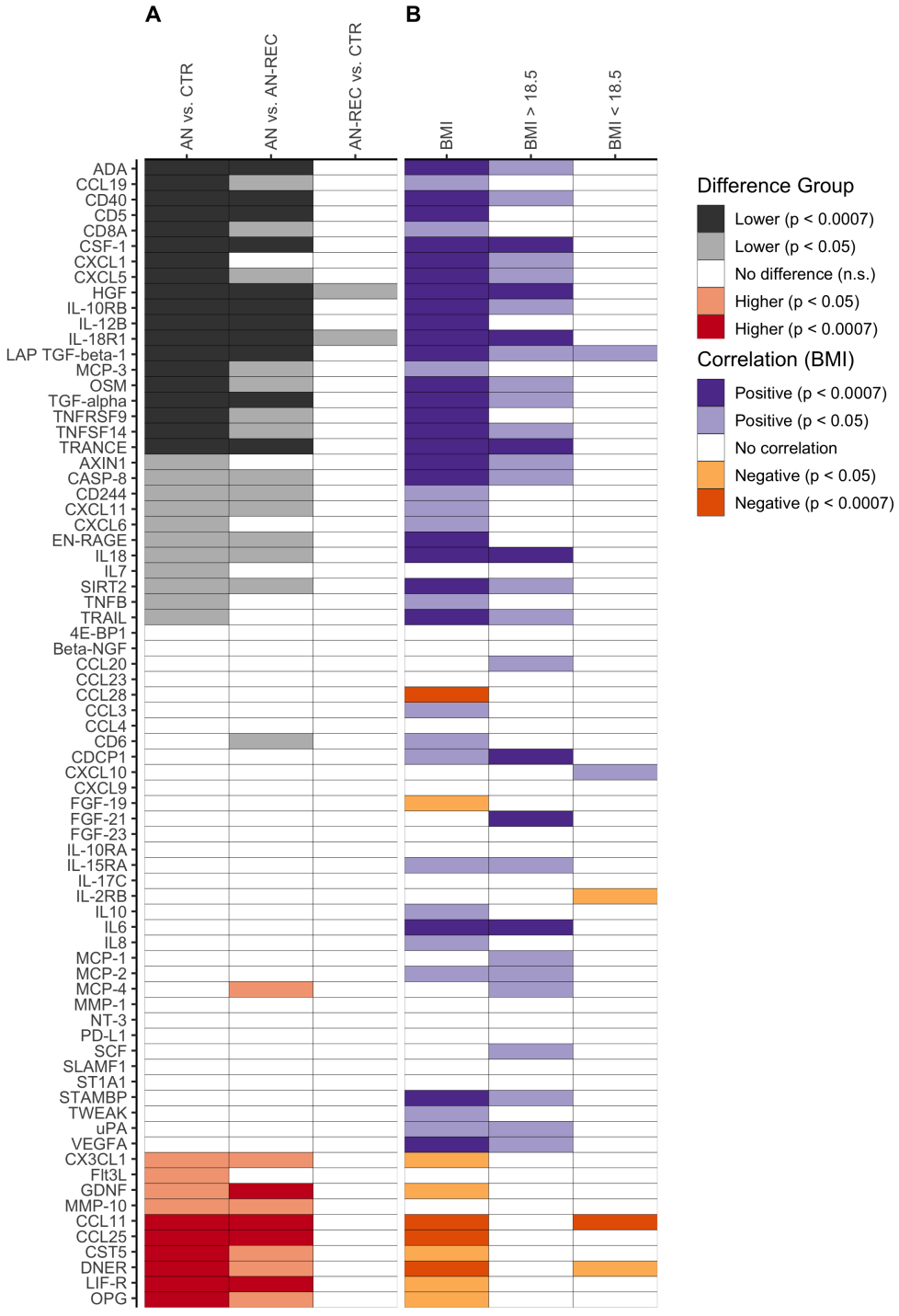

Figure 4.5: Plasma protein concentrations changed in AN vs. controls, AN vs. AN-REC, AN-REC vs. controls (A), associations between plasma protein concentrations and BMI (B). Colors indicate the direction of change, intensities the statistical significance. AN = anorexia nervosa, AN-REC = recovered from anorexia nervosa, CTR = normal weight healthy control.

patients with active AN, but not in patients that have recovered from AN. In parallel, we report that many of the inflammatory proteins correlate with BMI, corroborating several previous findings, but also bringing forward new ones. Even if the causal relationship is still not completely established, we hypothesize that normalizing the inflammatory profile in AN is desirable to reduce long-term effects, underlining the importance of providing fast and adequate treatment to patients with the disorder.

We are currently planning to follow-up the results of this study in a two-fold way. First, we will combine our dataset with PRS of AN, BMI, as well as the available PRS of marker levels. Finding associations between the genetic liability of AN/BMI and marker levels, or the genetic liability for increased levels of inflammatory markers and AN could allow us to draw conclusion on the directionality of the associations reported in this study. Furthermore, we are planning to measure the same panel in a longitudinal cohort including patients with AN during treatment. This will allow us to determine the exact timeline of protein changes, as well as testing whether more severe changes in inflammatory markers are associated with a slower recovery.

4.6 *In vitro* models in psychiatry

One aim of my PhD education was to establish and use cellular models of psychiatric disorders. This part of the project included (1) the taking of skin biopsies and derivation of fibroblast lines from patients; (2) the establishment of induced pluripotent stem cell lines from patients; (3) the derivation of neural stem cell lines from patients that could (4) finally be used as *in vitro* models of lithium response in BD. We were able to establish several protocols (including biopsy collection and processing, iPSC derivation, NSC derivation), as well as the development of new protocols (including serum-free culture of fibroblasts and simple organoid usage). However, much of the data is not yet in manuscript form and this project will be continued in collaboration with several other groups. In this section, I will present a short summary of the aims and the achieved results.

4.6.1 Fibroblast collection and establishment of iPSC lines

We established the collection of skin biopsies at Psychiatry Southwest, Karolinska University Hospital Huddinge, with the aim to collect 20 patients, 10 lithium-responders and 10 non-responders. We screened for patients in the bipolar cohort (cf. 3.1.1), based on Alda-scores. Inclusion criteria were BD type 1 and a family history of BD or schizophrenia. Lithium responders were defined as having a total Alda score ≥ 9, lithium non-responders as having an Alda score A ≤ 3. The recruitment process was considerably longer than expected until 17 sex-matched patients that met the criteria (8 responders,

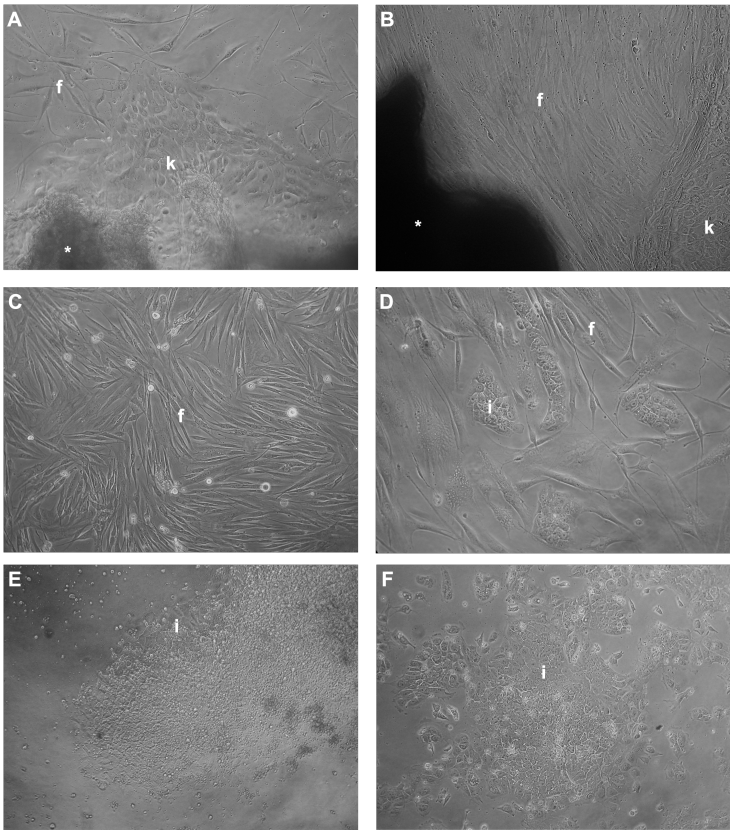

Figure 4.6: Examples of the steps in establishing fibroblast and iPSC lines. (A) Day 8 after plating the biopsies: After keratinocytes, the first fibroblasts migrate out of the biopsy. (B) Day 14: Fibroblasts form a confluent layer around the biopsy. (C) Fibroblasts after the first splitting. (D) Day 10 after transfection: The first iPSC colonies appear. (E) Day 2 after picking the colony. (F) Day 8 after picking the colony. Legend: *: biopsy, k: keratinocyte, f: fibroblasts, i: iPSC.

9 non-responders) were included. Although exact age matching was not possible, we obtained samples with similar age distribution.

Fibroblast lines were derived as described in section 3.5.1, expanded and three vials of each patient were cryopreserved (Fig. 4.6). We then established the protocol to derive iPSC in the lab (cf. 3.5.2) and were able to derive iPSC from a couple of patients (Fig. 4.7).

4.6.2 Development of a serum free fibroblast culture medium

A secondary aim was to find a metabolic signature for lithium response using mass spectrometry on samples obtained from untransformed fibroblasts of lithium responders and non-responders.

In order to achieve this, we first had to develop a serum-free and chemically defined medium to culture skin fibroblasts: Indeed, FBS, which is commonly used for fibroblast culture,

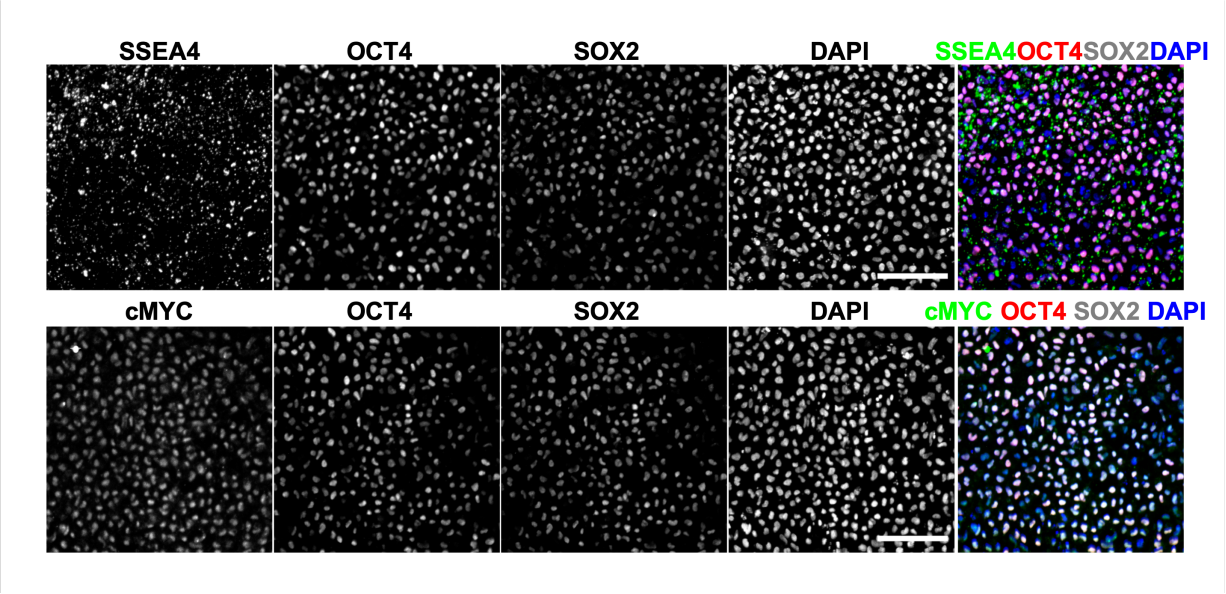

Figure 4.7: Basic characterization of one iPSC line by immunofluorescence, staining for the pluripotency markers OCT4, SOX2, c-MYC and SSEA4, nuclear staining using DAPI. Scale: 100μm.

represents an important obstacle in mass-spectrometric analysis, due to the high levels of proteins and metabolites present. We were able to define a protocol to adapt human skin fibroblasts to serum-free conditions (*data not shown, manuscript under review*). Although the proliferative potential of the cells was significantly reduced, the fibroblasts survived up to 6 weeks under these conditions. Furthermore, we were able to show that significant differences could be detected using mass-spectrometry when comparing the supernatant after three days in contact with the cells with fresh medium. This proof of concept confirmed active metabolism in cells, as well as the robustness of the cell medium for analyses using mass-spectrometry.

We then applied the protocol on the 17 patient fibroblast cell lines. In a first step, we adapted 6 wells of a 6-well plate to the serum-free medium, followed by a 2-week period where half the wells were treated with 1 mmol/L of lithium, and half were not. After 2 weeks, the medium was not changed for 3 days in order to increase the concentration of secreted metabolites. The supernatant, as well as the cells were then collected and snap-frozen.

The samples were then sent to the San Raffaele Scientific Institute in Milan for mass spectrometric analyses of the exo- and the endometabolome. However, due to technical delays, the final results have not been obtained yet.

4.6.3 Establishing neural stem cells

The third subproject was the generation of NSC. We were able to establish a protocol for NSC derivation and derive and characterize NSC from a human embryonic stem cell line (hES), as well as from iPSC. Characterization included karyotyping and gene expression analysis of the NSC, pluripotent and NSC marker analysis at protein level. Furthermore, we analysed the differentiation potential of NSC into neural and glial cells, as well as neuronal activity in mature neurons using electrophysiology (*data not shown, manuscript in preparation*). Using this protocol, we studied the effects of short-chain fatty acids on NSC and were able to show that treatment with acetate, propionate and butyrate at physiological levels led to an increase in cell proliferation[286]. The NSC are currently used by collaborators in two further projects.

4.6.4 Concluding remarks and future plans

This part of the project was an important part of the education and had several positive outcomes. In particular, I increased my knowledge on cell culture (fibroblast, hES, iPSC, NSC), molecular techniques, and, most importantly on planning and performing cell studies in psychiatry. As co-supervisor of two master students working on the projects, I got experience in teaching and supervision. Finally, the work on these projects led to the writing of a book chapter on cell models of mood disorders in an upcoming book on translational psychiatry.

Several manuscripts are in preparation and will be published in the coming year. Regarding the use of the patient derived cells, we are currently planning to start collaborations with established actors in the field.

Chapter 5

Concluding remarks and future perspectives

5.1 Concluding remarks

There are three themes covered by the studies included in the thesis that I will highlight and discuss: *replication*, *integration* and *translation*.

5.1.1 Replication

Replication is the gold standard by which scientific claims can be tested. If results are based on an effect that is real, it should be possible to replicate them, as long as similar procedures are used in cohorts with adequate power.

There is a replicability crisis affecting psychological and biomedical sciences[287,288], and several solutions have been suggested to tackle it, for example using lower threshold for p-values instead of the widely accepted α=0.05, or abandoning the concept all together[289]. Even if, in psychiatric genetics in particular, the use of more conservative threshold has led to clear improvements, replication remains an important tool in the advancement of science.

Several of the presented studies touch on the subject. *Study 1* is a direct replication study of previously published results. In *study 3*, we were able to replicate previously published results, as well as validate our findings in two separate cohorts, providing replication within the study. The genetic findings are however in need of replication, which we are aiming for in our future plans. We will test the explanatory power of PRS built on the cohorts separately, as well as aim at replicating the findings in a larger cohort in collaboration with ConLiGen. Finally, *study 4* is also based on a discovery and a replication cohort.

Regarding *study 2*, although support for the eQTL came from two different sources, the main findings require replication in larger cohorts. The importance of such replication studies became particularly clear when writing a systematic review about *SLC1A2*/EAAT2 in mood disorders. Although there has been a large amount of research on the subject, few studies report replications, meta-analysis was not possible and the overall role of the gene in mood disorders remains to be determined[290].

A common problem that arises is the difficulty to publish replication studies and negative results, replication studies often being published in journals with much lower impact than the originally reported results[291,292]. One consequence is the commonly known publication bias, which makes the interpretation of results more difficult.

5.1.2 Integration and collaboration

Several forms of *integration* have been important for this thesis, including integration of methods, cohorts and research fields.

The advantages of combining and integrating cohorts have been mentioned in several previous sections. The fact that our research group has been contributing data to both the Bipolar Working Group of the PGC and ConLiGen for several years has shown me the importance and advantages of collaborations and how they can drive science forward. Being part of it feels of utmost importance.

At a smaller scale, we have aimed at doing this in *study 2*, by combining our efforts with researchers from the Mayo Clinic, in *study 3*, with the researchers from the Department of Medical Epidemiology and Biostatistics around the BipoläR register, in *study 4*, with the ANGI-SE cohort, which finally also enabled *study 5*. We are planning to follow a similar path for our *cell studies*, where we are currently exploring possibilities in joining a larger collaboration.

Another form of integration that enabled many of the studies was the discussion across research field borders. Indeed, the NfL project was empowered by the close collaboration with researchers from the multiple sclerosis field, in which NfL has for a long time been established as biomarker. Many statistical methods found their way into my studies through discussion with researchers with psychology backgrounds and from the PET group, much more used working on Bayesian statistics, cross-validation and modelling[17].

Finally, the integration of several methods has been important in the thesis. While maybe not allowing for particular specialization, a basic understanding of diverse methodologies might be an advantage for an aspiring clinician-scientist interested in translational research.

5.1.3 Translation

Translational approaches were important in all studies and discussed previously. However, two aspects should be highlighted.

Studies 4 and 5 are examples of the translation from bench-to-bedside, as the research interest leading to the main hypotheses mainly originated in phenotypes observed in the *anx/anx* mouse model. Although our research approach allowed us only partially to translate the findings in mice, as blood can only reflect processes happening in the CNS in a limited way, our studies clearly indicate the potential of weaving together pre-clinical and clinical findings.

Study 3 exemplifies translation in the opposite direction, going from bedside-to-bench. Indeed, I became aware of the problem of lithium dosage while working in the clinics. The results from the genetic study do not point towards a single target that could increase the prediction potential enough to be used in clinics. However, if the predictive validity of a PRS based on our results is validated in future studies, one could imagine that with genotyping becoming more frequent, this PRS could be used in clinical practice. Until then, we will drive the translation from bench-to bedside forward, by testing the prediction algorithm based on clinical variables in a clinical setting.

5.2 Future perspectives

The field of translational psychiatry is growing rapidly. With results from different methodologies getting more robust and interpretable, it is only a matter of time until new concepts will be translated into clinical application. In regards to the results presented in this thesis, many of the studies have not reached their endpoints.

Specifically, *study 3* will be driven forward on two different paths: On one hand the clinical testing and possible implementation of the algorithm, on the other the strengthening of the genetic results. The first steps for validation of the algorithm have already been laid out, with information currently being collected in a prospective manner. If the results are promising, it may be part of a bigger project of digital health in BD that our group is currently working on. Regarding genetics, we are proposing to use the ConLiGen to obtain more data. If this second wave of GWAS provides stronger evidence, the genetic path could return to the clinic, with a possible implementation of genetic testing before lithium treatment.

The results of *study 4* and *study 5* will be followed up in a two-fold way, measuring the same markers in CSF, as well as in a longitudinal cohort. Here, we will continue to closely work together with our collaborators, integrating our efforts in regards to samples, financing and data analysis. The aim will be a better understanding of causal relationships between the

neurodegenerative and inflammatory pathways and AN, specifically in regards to clinical variables, specific behaviors, subtypes and outcome.

Finally, in regards to our cell culture project, we are currently planning to start collaborations with established actors in the field, to finish the project in BD, and possibly making our samples available to larger consortia. At the same time, a new collection of fibroblasts of patients with AN is currently being started. Guided by the experiences of our initial BD study, we are planning this project in a highly collaborative way to increase our chances of obtaining meaningful results.

5.2.1 A bridge to somewhere...

With so many open questions and opportunities, a lot of work still lies ahead. Having acquired a broad panel of research tools, I will do my best to take part in the building of future bridges.

Acknowledgments

It is an open secret that the acknowledgments are one of the most read sections of a thesis. For good reasons, as it is maybe the most important one, painting the right picture of the whole support network that made the thesis possible. It is the list of all co-authors without whom none of it would have been possible, the *et al.* missing on the cover.

So thank you, dear reader, for all the help and support you have given me! Do not blush if you have opened my thesis and jumped directly to this chapter, I have often done so myself.

With this, I would like to begin by thanking my main supervisor *Martin Schalling* who guided me on difficult paths (Fig. 5.1) and whose support and guidance made me grow both scientifically and personally. Thank you, for having given me so much freedom to explore, write, apply, travel and teach; for giving me a home; for never refusing an idea (even if you could have), always supporting and encourage me in the paths I chose, while helping me to adapt my research strategies, and from day one, considering me as a full member of the research group. I want to thank *Catharina Lavebratt*, my main co-supervisor. Thank you for having been there and taking responsibility every single time, I needed you; for giving clear lines and pushing me when needed, while always being patient with me; teaching me when I did not know, but always leaving me free to choose my ways. To both of you: I was very lucky having you as a team to supervise me. Without you, I would not be here today and I am looking forward to our future collaborations.

I would like to thank my other supervisors, *Carlos Villaescusa* for opening the beautiful world of stem cells to me, for all the technical and moral advice, and giving feedback and guiding me, even from the distance; *Sophie Erhardt* for her support and motivation, in projects past, present and future that have not found their way into this book, and *Robert Schwarcz* for always being there when needed. Even if I strolled away from the original path, inflammation and psychosis have made it back into the book. And the kynurenine pathway will always stay close to my heart.

Even if the list of official supervisors stops there, several *project supervisors* have played important roles in my education and I cannot thank them enough: *Ida Nilsson*, for introducing me to the field of anorexia nervosa, as well as to Swedish food culture, for all our collaborations and all the scientific and mental support; *Sarah Bergen* for her time,

Figure 5.1: Student observing the supervisor's path and wondering whether to take it.

her office and her great support the genome-wide analysis study; *Lena Backlund* for all the clinical knowledge and the access to the clinical materials, and *Granville Matheson*, friend, turned co-author, turned supervisor, for introducing me to wonderful new fields in statistics, data analysis and critical thinking. I also want to thank *Mikael Landén* whose participation was crucial for several studies, and whose feedback was always greatly appreciated. I am looking forward to continue collaborating with all of you.

I want to thank our clinical collaborators, *Lina Martinsson*, *Inger Römer-Ek*, *Katja Ponzer*, *Bonita Palm*, *Fredrik Wikström* and *Martin Lundberg*, who it was and will be a joy to work with. The only regret is not working longer next to you on the other side of the bridge.

I further would like to thank my mentor, *Anna Fogdell-Hahn*, as well as *Tomas Ekström* for all the discussions, thoughts, ideas and pieces of knowledge appearing over lunch or a coffee. Thank you for all your advice and guidance!

I want to extend my thanks to my fellow PhD-students and friends in the lab who have been my daily companions for the last years: *Mikael Ringh*, for having always been there six months in advance, serving as a guide in a big brother kind of way, and even providing a bed for me during the last period of this PhD. *Sahl Khalid Bedri*, for always reminding me to iron my shirts and to never take life too seriously. *Parvin Kumar* for all your random facts and all discussions, collaborations and travels that we have done together. *Nicky Dunn*, for never revealing your true name to me, and all the skiing (on water and snow) you made me do; *Liu Yang* for being the best desk neighbor anyone could imagine, for all your help and all the good results we have produced together; *Paschalis Efstathopoulos*, for your wisdom and wide knowledge regarding cell culture, for all after-works and for giving me the opportunity to visit Crete, holidays that changed my life; *Miranda Stiernborg*, for

you bright ideas, good mood and for always telling me how *duktig* I am; and *Linghua Kong*, for being a source of calm and an example of hard work and dedication.

To my friends and fellow PhD-students outside of the lab, *Susanne Neumann* and *Suzanna Queckbörner*, who went with me through *dick und dünn*, quite literally, and who shared so many moments with me. I would not want to imagine, what my time in Stockholm would have been without you and I am very pleased that I do not have to. Also *Pontus Plaven Sigray*, for inspiring me to create a cake and for all passionate discussions about everything, *Henna Salo*, for your dry humor, *Natalie Sippl*, for your positive attitude and happiness, *Lara Kular*, for being my French friend, *Johanna Wolfesberger*, for being my Austrian friend (both positions are needed, and very important), *Ewoud Ewing* and *Sanjay Fernandes*, for having always been there, from the beginning to the end. A big thanks to the *stats nerds*, *Mimmi Lee*, *Joachim Brumberg*, and *Gina Griffioen*, for all the fun we had and will have, discussing waffles and tulips, and to *Vera Kerstens* and *Moa Pontén* for the *Gassenhauer*.

A big thanks goes to the master student's I supervised, *Jakob Schuy*, *Laura Baqué Vidal* and *Iris Garcia Alcantarilla*. I have probably learned much more from you than you from me, and it was a pleasure to work with you. I am glad to have been able to participate in your growth and even happier that you have found places where you will develop so much further!

I would also like to thank the members of KI-Gene, *Annika Eriksson*, *Katharina Gell* and *Malin Alvehus*, as well as all the other *people of L8:00*, present and past, *Anna Matsson*, *Nastya Kharlamova*, *Elin Engdahl*, *Christina Hermanrud*, *Malin Ryner*, *Virginija Danylaité Karrenbauer*, *Ingegerd Löfving Arvholm*, *Malin Almgren*, *Lollo Sjöholm*, *Joelle Ruegg*, *Annika Lindblom*, *Anna Witasp*, *Dzana Hukic*, *Urban Ösby*, *Karin Lutropp*, *Philippe Melas*, *Anna-Lee Köstinger*, *Selim Sengul*, *Xinxia Chen*, *Yabin Wei*, *Jiajia Liu*, *Ozsvár Judit*, *Paulina Łuków*, *Claire Thume*, *Elizabeta Zaplatic*, *Yujiao Wu*, *Jingya Yu*, *Xiaotian Yan* and all members of the *Hematology group*, for creating an environment that was always pleasant personally and inspiring scientifically. Thanks to you I was happy to come to work (almost) every single day during these fast years.

I want to thank all researchers from the Mayo Clinic who have welcomed me open-heartedly, taught me and integrated me, *Marin Veldic*, *Mark Frye*, *Jacquetta Blacker*, *Joanna Biernacka* and *Brandon Coombes*. Thank you for the stays, the collaborations that we did and all that we are still to do. A big thank you to *Cynthia Bulik* and *Christopher Huebl* for their support in the studies on anorexia nervosa, for being inspirations and examples to follow; and to all members of Sophie's and *Göran Engberg*'s groups, *Lilly Schwieler*, *Anthi Faka*, *Funda Orhan*, *Maximilian Tufvesson Alm*, *Michel Goiny* and *Sophie Imbeault*, for accepting me as one of your own during a few months, for all support and scientific exchanges, past and future. My thanks also goes to all *other co-authors*, as well as all *patients that have agreed in participating in our studies*, without whom nothing would have been possible.

Thank you to all members of the MMK and CMM administration and IT-department, in particular *Ann-Britt Wikström*, *Britt-Marie Witasp*, *Annika Hederby*, *Timmy Hellström*, *Christer Uvehag* and *Hugo Åberg*, for all the support and help you have given me, and making the whole doctoral education run so smoothly.

To all my friends that I (re)made here, *Leo*, *Joachim*, *Zara*, *Sazia*, *Gabriel*, *Felicia*, *Sebastian* and *Adam*, thank you for all the parties, dinners, excursions and discussions over all these years, which made me really feel at home in Stockholm! To all my friends abroad, *Mimi* and *Flo*, for their support from the British Isles; *Simon* and *Charlotte* from the APHP; *Julian* and *Sascha* from multiple locations; *Karo*, *Jakob*, *Bernadette*, and all my friends that are keeping Vienna warm. To all my teachers and mentors, I had over the years, *Yonnel*, *Luca*, *Joseph*, *Christoph*, *David*, *Maria*, *Thérèse*, *Boris* and *Otto*, for all the ground work you have laid and all the knowledge you have given me.

Coming to the last authors on this list, an infinite amount of thank you to my family. To my fiancée *Irene*, who has been by my side, supporting and loving from the first day we met, from the chocolate for dinner, after running late in the lab on a Saturday evening; to the numerous weekends spent with me in the lab while feeding the cells; to the drawing of the cover of my thesis and the moral support during the final weeks. You have been the best partner I could have ever imagined, and I am blessed to have you in my life!

To my parents, *Margret* and *Christophe*, who have always supported and cared for me, loved me from near and far, trembled and cheered with me, and given me the absolute security net any child could dream of. I hope I could make you a bit proud. And to my brother and life-long example *Laurent* and my sister-in-law *Juliette* for having always been supportive and loving, and in particular during these last years, making me live in the security, that you will always be here for me.

Appendix A

Diagnostic criteria

A.1 Bipolar disorder

DSM-V[30]

Bipolar I

For a diagnosis of bipolar I disorder, it is necessary to meet the following criteria for a manic episode. The manic episode may have been preceded by and may be followed by hypomanic or major depressive episodes.

Bipolar II

For a diagnosis of bipolar II disorder, it is necessary to meet the following criteria for a current or past hypomanic episode and the following criteria for a current or past major depressive episode:

Manic episode

1. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy, lasting at least 1 week and present most of the day, nearly every day (or any duration if hospitalization is necessary).
2. During the period of mood disturbance and increased energy or activity, three (or more) of the following symptoms (four if the mood is only irritable) are present to a significant degree and represent a noticeable change from usual behavior:

    - Inflated self-esteem or grandiosity.
    - Decreased need for sleep.
    - More talkative than usual or pressure to keep talking.
    - Flight of ideas or subjective experience that thoughts are racing.

- Distractibility.
- Increase in goal-directed activity or psychomotor agitation.
- Excessive involvement in activities that have a high potential for painful consequences.

3. The mood disturbance is sufficiently severe to cause marked impairment in social or occupational functioning or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.

4. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or another medical condition.

Hypomanic episode

The differences between a manic and a hypomanic episode are in *italic*.

1. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy, lasting *at least 4 consecutive days* and present most of the day, nearly every day.

2. cf. Manic episode

3. *The episode is associated with an unequivocal change in functioning that is uncharacteristic of the individual when not symptomatic.*

4. *The disturbance in mood and the change in functioning are observable by others.*

5. *The episode is not severe enough to cause marked impairment in social or occupational functioning or to necessitate hospitalization. If there are psychotic features, the episode is, by definition, manic.*

6. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or another medical condition.

Major depressive episode

1. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. +Depressed mood +Markedly diminished interest in all or almost all activities +Weight loss or weight gain +Insomnia or hypersomnia +Psychomotor agitation or retardation +Fatigue or loss of energy +Feelings of worthlessness or excessive or innapropriate guilt +Diminished ability to think or concentrate +Recurrent thoughts of death, suicidal ideation or a suicide attempt

2. The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning.

3. The episode is not attributable to the physiological effects of a substance or another medical condition.

Specifier: With rapid cycling

Presence of at least four mood episodes in the previous 12 months that meet the criteria for manic, hypomanic, or major depressive episode.

Note: Episodes are demarcated by either partial or full remissions of at least 2 months or a switch to an episode of the opposite polarity (e.g., major depressive episode to manic episode).

Note: The essential feature of a rapid-cycling bipolar disorder is the occurrence of at least four mood episodes during the previous 12 months. These episodes can occur in any combination and order. The episodes must meet both the duration and symptom number criteria for a major depressive, manic, or hypomanic episode and must be demarcated by either a period of full remission or a switch to an episode of the opposite polarity. Manic and hypomanic episodes are counted as being on the same pole. Except for the fact that they occur more frequently, the episodes that occur in a rapid-cycling pattern are no different from those that occur in a non-rapid-cycling pattern. Mood episodes that count toward defining a rapid-cycling pattern exclude those episodes directly caused by a substance (e.g., cocaine, corticosteroids) or another medical condition.

Specifier: With psychotic features

Delusions or hallucinations are present at any time in the episode. If psychotic features are present, specify if mood- congruent or mood-incongruent:

When applied to current or most recent manic episode (in bipolar I disorder):

With mood-congruent psychotic features: The content of all delusions and hallucinations is consistent with the typical manic themes of grandiosity, invulnerability, etc., but may also include themes of suspiciousness or paranoia, especially with respect to others' doubts about the individual's capacities, accomplishments, and so forth.

With mood-incongruent psychotic features: The content of delusions and hallucinations does not involve typical manic themes as described above, or the content is a mixture of mood-incongruent and mood-congruent themes.

When applied to current or most recent major depressive episode (in bipolar I disorder or bipolar II disorder):

With mood-congruent psychotic features: The content of all delusions and hallucinations is consistent with the typical depressive themes of personal inadequacy, guilt, disease, death, nihilism, or deserved punishment.

With mood-incongruent psychotic features: The content of the delusions or hallucinations does not involve typical depressive themes of personal inadequacy, guilt, disease, death, nihilism, or deserved punishment, or the content is a mixture of mood-incongruent and mood- congruent themes.

ICD-10[6]

F30.0 - Hypomania A disorder characterized by a persistent mild elevation of mood, increased energy and activity, and usually marked feelings of well-being and both physical and mental efficiency. Increased sociability, talkativeness, over-familiarity, increased sexual energy, and a decreased need for sleep are often present but not to the extent that they lead to severe disruption of work or result in social rejection. Irritability, conceit, and boorish behaviour may take the place of the more usual euphoric sociability. The disturbances of mood and behaviour are not accompanied by hallucinations or delusions.

F30.1 - Mania without psychotic symptoms Mood is elevated out of keeping with the patient's circumstances and may vary from carefree joviality to almost uncontrollable excitement. Elation is accompanied by increased energy, resulting in overactivity, pressure of speech, and a decreased need for sleep. Attention cannot be sustained, and there is often marked distractibility. Self-esteem is often inflated with grandiose ideas and overconfidence. Loss of normal social inhibitions may result in behaviour that is reckless, foolhardy, or inappropriate to the circumstances, and out of character.

F30.2 - Mania with psychotic symptoms In addition to the clinical picture described in F30.1, delusions (usually grandiose) or hallucinations (usually of voices speaking directly to the patient) are present, or the excitement, excessive motor activity, and flight of ideas are so extreme that the subject is incomprehensible or inaccessible to ordinary communication.

F31. - Bipolar affective disorder (BAD) A disorder characterized by two or more episodes in which the patient's mood and activity levels are significantly disturbed, this disturbance consisting on some occasions of an elevation of mood and increased energy and activity (hypomania or mania) and on others of a lowering of mood and decreased energy and activity (depression). Repeated episodes of hypomania or mania only are classified as bipolar.

F31.0 - BAD, current episode hypomanic The patient is currently hypomanic, and has had at least one other affective episode (hypomanic, manic, depressive, or mixed) in the past.

F31.1 - BAD, current episode manic without psychotic symptoms The patient is currently manic, without psychotic symptoms (as in F30.1), and has had at least one other affective episode (hypomanic, manic, depressive, or mixed) in the past.

F31.2 - BAD, current episode manic with psychotic symptoms The patient is currently manic, with psychotic symptoms (as in F30.2), and has had at least one other affective episode (hypomanic, manic, depressive, or mixed) in the past.

F31.3 - BAD, current episode mild or moderate depression The patient is currently depressed, as in a depressive episode of either mild or moderate severity (F32.0 or F32.1), and has had at least one authenticated hypomanic, manic, or mixed affective episode in the past.

F31.4 - BAD, current episode severe depression without psychotic symptoms The patient is currently depressed, as in severe depressive episode without psychotic symptoms (F32.2), and has had at least one authenticated hypomanic, manic, or mixed affective episode in the past.

F31.5 - BAD, current episode severe depression with psychotic symptoms The patient is currently depressed, as in severe depressive episode with psychotic symptoms (F32.3), and has had at least one authenticated hypomanic, manic, or mixed affective episode in the past.

F31.6 - BAD, current episode mixed The patient has had at least one authenticated hypomanic, manic, depressive, or mixed affective episode in the past, and currently exhibits either a mixture or a rapid alteration of manic and depressive symptoms.

F31.7 - BAD, currently in remission The patient has had at least one authenticated hypomanic, manic, or mixed affective episode in the past, and at least one other affective episode in addition, but is not currently suffering from any significant mood disturbance, and has not done so for several months.

F32 - Depressive episode In typical mild, moderate, or severe depressive episodes, the patient suffers from lowering of mood, reduction of energy, and decrease in activity. Capacity for enjoyment, interest, and concentration is reduced, and marked tiredness after even minimum effort is common. Sleep is usually disturbed and appetite diminished. Self-esteem and self-confidence are almost always reduced and, even in the mild form, some ideas of guilt or worthlessness are often present. The lowered mood varies little from day to day, is unresponsive to circumstances and may be accompanied by so-called "somatic" symptoms, such as loss of interest and pleasurable feelings, waking in the morning several hours before the usual time, depression worst in the morning, marked psychomotor retardation, agitation, loss of appetite, weight loss, and loss of libido. Depending upon the number and severity of the symptoms, a depressive episode may be specified as mild, moderate or severe.

F33 - Recurrent depressive disorder A disorder characterized by repeated episodes of depression as described for depressive episode (F32.-), without any history of independent episodes of mood elevation and increased energy (mania). There may, however, be brief episodes of mild mood elevation and overactivity (hypomania) immediately after a depressive episode, sometimes precipitated by antidepressant treatment. [...] The first episode may occur at any age from childhood to old age, the onset may be either acute or insidious, and the duration varies from a few weeks to many months. The risk that a patient with recurrent depressive disorder will have an episode of mania never disappears completely, however many depressive episodes have been experienced. If such an episode does occur, the diagnosis should be changed to bipolar affective disorder (F31.-).[6]

A.2 Anorexia Nervosa

DSM-V[7]

1. Restriction of energy intake relative to requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health. *Significantly low weight* is defined as a weight that is less than minimally normal or, for children and adolescents, less than that minimally expected.

2. Intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight.

3. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.

The ICD-10-CM code depends on the subtype. Specify whether:

1. **(F50.01) Restricting type**: During the last 3 months, the individual has not engaged in recurrent episodes of binge eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas). This subtype describes presentations in which weight loss is accomplished primarily through dieting, fasting, and/or excessive exercise.
2. **(F50.02) Binge-eating/purging type**: During the last 3 months, the individual has engaged in recurrent episodes of binge eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas).

Specify if:

1. **In partial remission**: After full criteria for anorexia nervosa were previously met, Criterion 1 (low body weight) has not been met for a sustained period, but either Criterion 2 (intense fear of gaining weight or becoming fat or behavior that interferes with weight gain) or Criterion 3 (disturbances in self-perception of weight and shape) is still met.
2. **In full remission**: After full criteria for anorexia nervosa were previously met, none of the criteria have been met for a sustained period of time.

Specify current severity: The minimum level of severity is based, for adults, on current body mass index (BMI) (see below) or, for children and adolescents, on BMI percentile. The ranges below are derived from World Health Organization categories for thinness in adults; for children and adolescents, corresponding BMI percentiles should be used. The level of severity may be increased to reflect clinical symptoms, the degree of functional disability, and the need for supervision.

- Mild: BMI >= 17 kg/m2
- Moderate: BMI 16–16.99 kg/m2
- Severe: BMI 15–15.99 kg/m2
- Extreme: BMI < 15 kg/m2

ICD-10[6]

F50.0 - Anorexia Nervosa A disorder characterized by deliberate weight loss, induced and sustained by the patient. It occurs most commonly in adolescent girls and young women, but adolescent boys and young men may also be affected, as may children approaching puberty and older women up to the menopause. The disorder is associated with a specific psychopathology whereby a dread of fatness and flabbiness of body contour persists as an intrusive overvalued idea, and the patients impose a low weight threshold on themselves. There is usually undernutrition of varying severity with secondary endocrine and metabolic changes and disturbances of bodily function. The symptoms include restricted dietary choice, excessive exercise, induced vomiting and purgation, and use of appetite suppressants and diuretics.

F50.1 - Atypical anorexia nervosa Disorders that fulfil some of the features of anorexia nervosa but in which the overall clinical picture does not justify that diagnosis. For instance, one of the key symptoms, such as amenorrhoea or marked dread of being fat, may be absent in the presence of marked weight loss and weight-reducing behaviour. This diagnosis should not be made in the presence of known physical disorders associated with weight loss.

References

All cited websites were accessed between November 2019 and February 2020 and were still accessible when the thesis went to print.

1. Cross-Disorder Group of the Psychiatric Genomics Consortium, Wellcome Trust Case-Control Consortium, Research team, Psychosis Endophenotypes International Consortium & others. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell* **179**, 1469–1482 (2019).

2. Ruderfer, D. M. *et al.* Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. *Cell* **173**, 1705–1715 (2018).

3. Mitelman, S. A. Transdiagnostic neuroimaging in psychiatry: A review. *Psychiatry Research* **277**, 23–38 (2019).

4. Coleman, J. R. I. *et al.* Genome-wide gene-environment analyses of major depressive disorder and reported lifetime traumatic experiences in UK biobank. *Molecular Psychiatry* (2020) doi:10.1038/s41380-019-0546-6.

5. Insel, T. R. A bridge to somewhere. *Translational psychiatry* **1**, e2 (2011).

6. World Health Organisation. *International Statistical Classification of Diseases and Related Health Problems (ICD)-10.* (1992).

7. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, Fifth Edition.* (2013).

8. Insel, T. Transforming Diagnosis. (2013).

9. National Institute of Mental Health. Research Domain Criteria (RDoC). (2017).

10. Gottesman, I. I. & Gould, T. D. The endophenotype concept in psychiatry: Etymology and strategic intentions. *American journal of psychiatry* **160**, 636–645 (2003).

11. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics* **69**, 89–95 (2001).

12. Nasrallah, H. A. Biomarkers in neuropsychiatric disorders: Translating research to clinical applications. *Biomarkers in Neuropsychiatry* **1**, 100001 (2019).

13. Flint, J. & Kendler, K. S. The Genetics of Major Depression. *Neuron* **81**, 484–503 (2014).

14. Duncan, L. E., Ostacher, M. & Ballon, J. How genome-wide association studies (gwas) made traditional candidate gene studies obsolete. *Neuropsychopharmacology* **44**, 1518–1523 (2019).

15. Sullivan, P. F. *et al.* Psychiatric genomics: An update and an agenda. *The American journal of psychiatry* **175**, 15–27 (2018).

16. Aydin, O., Aydin, P. U. & Arslan, A. Development of neuroimaging-based biomarkers in psychiatry. in *Frontiers in psychiatry* 159–195 (Springer Singapore, 2019). doi:10.1007/978-981-32-9721-0_9.

17. Matheson, G. J. *et al.* Clinical brain pet research must embrace multi-centre collaboration and data sharing or risk its demise. *European journal of nuclear medicine and molecular imaging* 1–3 (2019).

18. Yuan, N., Chen, Y., Xia, Y., Dai, J. & Liu, C. Inflammation-related biomarkers in major psychiatric disorders: A cross-disorder assessment of reproducibility and specificity in 43 meta-analyses. *Translational Psychiatry* **9**, (2019).

19. Teixeira, A. L., Salem, H., Frey, B. N., Barbosa, I. G. & Machado-Vieira, R. Update on bipolar disorder biomarker candidates. *Expert review of molecular diagnostics* **16**, 1209–1220 (2016).

20. Bai, S. *et al.* Efficacy and safety of anti-inflammatory agents for the treatment of major depressive disorder: A systematic review and meta-analysis of randomised controlled trials. *Journal of Neurology, Neurosurgery & Psychiatry* **91**, 21–32 (2019).

21. Soliman, M. A., Aboharb, F., Zeltner, N. & Studer, L. Pluripotent stem cells in neuropsychiatric disorders. *Molecular Psychiatry* 1–9 (2017) doi:10.1038/mp.2017.40.

22. Takahashi, K. *et al.* Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **131**, 861–872 (2007).

23. Staerk, J. *et al.* Reprogramming of Human Peripheral Blood Cells to Induced Pluripotent Stem Cells. *Cell Stem Cell* **7**, 20–24 (2010).

24. Brennand, K. J. *et al.* Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **473**, 221–225 (2011).

25. Mertens, J. *et al.* Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature* 1–17 (2015) doi:10.1038/nature15526.

26. Stern, S. *et al.* Neurons derived from patients with bipolar disorder divide into intrinsically different sub-populations of neurons, predicting the patients' responsiveness to lithium. *Molecular Psychiatry* 1–13 (2017) doi:10.1038/mp.2016.260.

27. Hoffman, G. E., Schrode, N., Flaherty, E. & Brennand, K. J. New considerations for hiPSC-based models of neuropsychiatric disorders. *Molecular psychiatry* **24**, 49–66 (2019).

28. Fromer, M. *et al.* Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nature Neuroscience* **19**, 1442–1453 (2016).

29. Warren, C. R., Jaquish, C. E. & Cowan, C. A. The nextgen genetic association studies consortium: A foray into in vitro population genetics. *Cell stem cell* **20**, 431–433 (2017).

30. American Psychiatric Association. Bipolar disorders. in *Diagnostic and statistical manual of mental disorders, fifth edition* (2013). doi:http://dx.doi.org/10.1176/appi.books.9780890425596.dsm03.

31. Vieta, E. *et al.* Bipolar disorders. *Nature reviews. Disease primers* **4**, 18008 (2018).

32. Abuse, S. & Mental Health Services Administration. *Impact of the dsm-iv to dsm-5 changes on the national survey on drug use and health [internet].* https://www.ncbi.nlm.nih.gov/books/NBK519697/ (2016).

33. Merikangas, K. R. *et al.* Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch.Gen.Psychiatry* **68**, 241–251 (2011).

34. Rowland, T. A. & Marwaha, S. Epidemiology and risk factors for bipolar disorder. *Therapeutic advances in psychopharmacology* **8**, 251–269 (2018).

35. Lee, S. *et al.* Rapid-cycling bipolar disorder: Cross-national community study. *The British Journal of Psychiatry* **196**, 217–225 (2010).

36. Goodwin, F. K. & Jamison, K. R. *Manic-depressive illness: Bipolar disorders and recurrent depression.* vol. 1 (Oxford University Press, 2007).

37. Schaffer, A. *et al.* Epidemiology, neurobiology and pharmacological interventions related to suicide deaths and suicide attempts in bipolar disorder: Part I of a report of the International Society for Bipolar Disorders Task Force on Suicide in Bipolar Disorder. *The Australian and New Zealand journal of psychiatry* **49**, 785–802 (2015).

38. Maletic, V. & Raison, C. Integrated Neurobiology of Bipolar Disorder. *Frontiers in Psychiatry* **5**, (2014).

39. Craddock, N. & Jones, I. Genetics of bipolar disorder. *J Med Genet* **36**, 585–594 (1999).

40. Craddock, N. & Sklar, P. Genetics of bipolar disorder. *The Lancet* **381**, 1654–1662 (2013).

41. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell* **179**, 1469–1482 (2019).

42. Watson, H. J. *et al.* Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. *Nature genetics* **51**, 1207–1214 (2019).

43. Seifuddin, F. *et al.* Meta-analysis of genetic association studies on bipolar disorder. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **159B**, 508–18 (2012).

44. Stahl, E. A. *et al.* Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nature genetics* **51**, 793–803 (2019).

45. Prata, D. P., Costa-Neves, B., Cosme, G. & Vassos, E. Unravelling the genetic basis of schizophrenia and bipolar disorder with gwas: A systematic review. *Journal of psychiatric research* (2019).

46. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427 (2014).

47. Green, E. K. *et al.* Copy number variation in bipolar disorder. *Molecular Psychiatry* **21**, 89–93 (2016).

48. Malhotra, D. & Sebat, J. CNVs: Harbingers of a Rare Variant Revolution in Psychiatric Genetics. *Cell* **148**, 1223–1241 (2012).

49. Bergen, S. E. *et al.* Genome-wide association study in a Swedish population yields support for greater CNV and MHC involvement in schizophrenia compared with bipolar disorder. *Molecular Psychiatry* **17**, 880–886 (2012).

50. Hibar, D. *et al.* Cortical abnormalities in bipolar disorder: An mri analysis of 6503 individuals from the enigma bipolar disorder working group. *Molecular psychiatry* **23**, 932–942 (2018).

51. Hibar, D. *et al.* Subcortical volumetric abnormalities in bipolar disorder. *Molecular psychiatry* **21**, 1710–1716 (2016).

52. Favre, P. *et al.* Widespread white matter microstructural abnormalities in bipolar disorder: Evidence from mega-and meta-analyses across 3033 individuals. *Neuropsychopharmacology* **44**, 2285–2293 (2019).

53. Gigante, A. D. *et al.* Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: A meta-analysis. *Bipolar disorders* **14**, 478–487 (2012).

54. Chitty, K. M., Lagopoulos, J., Lee, R. S. C., Hickie, I. B. & Hermens, D. F. A systematic review and meta-analysis of proton magnetic resonance spectroscopy and mismatch negativity in bipolar disorder.

European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology **23**, 1348–1363 (2013).

55. Pereira, L. P. *et al.* The relationship between genetic risk variants with brain structure and function in bipolar disorder: A systematic review of genetic-neuroimaging studies. *Neuroscience & Biobehavioral Reviews* **79**, 87–109 (2017).

56. Viswanath, B. *et al.* Cellular models to study bipolar disorder: A systematic review. *Journal of affective disorders* **184**, 36–50 (2015).

57. Jiang, X. *et al.* Sodium valproate rescues expression of trank1 in iPSC-derived neural cells that carry a genetic variant associated with serious mental illness. *Molecular psychiatry* **24**, 613–624 (2019).

58. Harrison, P. J., Colbourne, L. & Harrison, C. H. The neuropathology of bipolar disorder: Systematic review and meta-analysis. *Molecular psychiatry* 1–22 (2018).

59. Schmider, J. *et al.* Combined dexamethasone/corticotropin-releasing hormone test in acute and remitted manic patients, in acute depression, and in normal controls: I. *Biological Psychiatry* **38**, 797–802 (1995).

60. Murri, M. B. *et al.* The hpa axis in bipolar disorder: Systematic review and meta-analysis. *Psychoneuroendocrinology* **63**, 327–342 (2016).

61. Havermans, R., Nicolson, N. A., Berkhof, J. & deVries, M. W. Patterns of salivary cortisol secretion and responses to daily events in patients with remitted bipolar disorder. *Psychoneuroendocrinology* **36**, 258–265 (2011).

62. Grossman, F. & Potter, W. Z. Catecholamines in depression: A cumulative study of urinary norepinephrine and its major metabolites in unipolar and bipolar depressed patients versus healthy volunteers at the nimh. *Psychiatry research* **87**, 21–27 (1999).

63. Frye, M. A. *et al.* Association between lower serum free t4 and greater mood instability and depression in lithium-maintained bipolar patients. *The American journal of psychiatry* **156**, 1909–1914 (1999).

64. Modabbernia, A., Taslimi, S., Brietzke, E. & Ashrafi, M. Cytokine alterations in bipolar disorder: A meta-analysis of 30 studies. *Biological psychiatry* **74**, 15–25 (2013).

65. Munkholm, K., Braüner, J. V., Kessing, L. V. & Vinberg, M. Cytokines in bipolar disorder vs. Healthy control subjects: A systematic review and meta-analysis. *Journal of psychiatric research* **47**, 1119–1133 (2013).

66. Rowland, T. *et al.* Neurotrophins, cytokines, oxidative stress mediators and mood state in bipolar disorder: Systematic review and meta-analyses. *The British Journal of Psychiatry* **213**, 514–525 (2018).

67. Tylee, D. S. *et al.* Genetic correlations among psychiatric and immune-related phenotypes based on genome-wide association data. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* **177**, 641–657 (2018).

68. Milhiet, V., Etain, B., Boudebesse, C. & Bellivier, F. Circadian biomarkers, circadian genes and bipolar disorders. *Journal of Physiology-Paris* **105**, 183–189 (2011).

69. Milhiet, V. *et al.* Circadian abnormalities as markers of susceptibility in bipolar disorders. *Front Biosci (Schol Ed)* **6**, 120–137 (2014).

70. Rock, P., Goodwin, G., Harmer, C. & Wulff, K. Daily rest-activity patterns in the bipolar phenotype: A controlled actigraphy study. *Chronobiology international* **31**, 290–296 (2014).

71. Bae, M. *et al.* Lifetime experiences of hypomanic symptoms are associated with delayed and irregular sleep–wake cycle and seasonality in non-clinical adult samples. *Comprehensive psychiatry* **55**, 1111–1115 (2014).

72. Jones, S. H., Hare, D. J. & Evershed, K. Actigraphic assessment of circadian activity and sleep patterns in bipolar disorder. *Bipolar disorders* **7**, 176–186 (2005).

73. Gonzalez, R. The relationship between bipolar disorder and biological rhythms. *The Journal of clinical psychiatry* **75**, e323–31 (2014).

74. Morris, G. *et al.* A model of the mitochondrial basis of bipolar disorder. *Neuroscience & Biobehavioral Reviews* **74**, 1–20 (2017).

75. Cade, J. F. Lithium salts in the treatment of psychotic excitement. *The Medical Journal of Australia* **II**, 349–350 (1949).

76. Joas, E. *et al.* Pharmacological treatment and risk of psychiatric hospital admission in bipolar disorder. *The British journal of psychiatry : the journal of mental science* **210**, 197–202 (2017).

77. Grandjean, E. M. & Aubry, J.-M. Lithium: Updated human knowledge using an evidence-based approach: Part iii: Clinical safety. *CNS drugs* **23**, 397–418 (2009).

78. Song, J. *et al.* Suicidal behavior during lithium and valproate treatment: A within-individual 8-year prospective study of 50,000 patients with bipolar disorder. *The American journal of psychiatry* **174**, 795–802 (2017).

79. Alda, M. Lithium in the treatment of bipolar disorder: pharmacology and pharmacogenetics. *Molecular Psychiatry* 1–10 (2015) doi:10.1038/mp.2015.4.

80. Malhi, G. S., Tanious, M., Das, P., Coulston, C. M. & Berk, M. Potential mechanisms of action of lithium in bipolar disorder: Current understanding. *CNS Drugs* **27**, 135–153 (2013).

81. Malhi, G. S. & Outhred, T. Therapeutic Mechanisms of Lithium in Bipolar Disorder: Recent Advances and Current Understanding. *CNS Drugs* **30**, 931–949 (2016).

82. Caberlotto, L. *et al.* Differential effects of glycogen synthase kinase 3 (gsk3) inhibition by lithium or selective inhibitors in the central nervous system. *Naunyn-Schmiedeberg's archives of pharmacology* **386**, 893–903 (2013).

83. Ward, M. E., Musa, M. N. & Bailey, L. Clinical pharmacokinetics of lithium. *Journal of clinical pharmacology* **34**, 280–285 (1994).

84. Diamond, J. M., Ehrlich, B. E., Morawski, S. G., Santa Ana, C. A. & Dordtran, J. S. Lithium absorption in tight and leaky segments of intestine. *The Journal of membrane biology* **72**, 153–159 (1983).

85. Grandjean, E. M. & Aubry, J.-M. Lithium: Updated human knowledge using an evidence-based approach. *CNS drugs* **23**, 225–240 (2009).

86. Soares, J. C., Boada, F. & Keshavan, M. S. Brain lithium measurements with 7Li magnetic resonance spectroscopy (mrs): A literature review. *European Neuropsychopharmacology* **10**, 151–158 (2000).

87. Smith, F. E. *et al.* 3D 7 li magnetic resonance imaging of brain lithium distribution in bipolar disorder. *Molecular psychiatry* **23**, 2184–2191 (2018).

88. Koomans, H. A., Boer, W. H. & Mees, E. J. D. Evaluation of lithium clearance as a marker of proximal tubule sodium handling. *Kidney international* **36**, (1989).

89. Mason, R., McQueen, E., Keary, P. & James, N. M. Pharmacokinetics of lithium: Elimination half-time, renal clearance and apparent volume of distribution in schizophrenia. *Clinical pharmacokinetics* **3**, 241–246 (1978).

90. Thornhill, D. & Field, S. Distribution of lithium elimination rates in a selected population of psychiatric patients. *European journal of clinical pharmacology* **21**, 351–354 (1982).

91. Hewick, D. S., Newbury, P., Hopwood, S., Naylor, G. & Moody, J. Age as a factor affecting lithium therapy. *British journal of clinical pharmacology* **4**, 201–205 (1977).

92. Reiss, R. A. *et al.* Lithium pharmacokinetics in the obese. *Clinical Pharmacology & Therapeutics* **55**, 392–398 (1994).

93. Goodnick, P. J., Fieve, R. R., Meltzer, H. L. & Dunner, D. L. Lithium elimination half-life and duration of therapy. *Clinical Pharmacology & Therapeutics* **29**, 47–50 (1981).

94. Oedegaard, K. J. *et al.* The pharmacogenomics of bipolar disorder study (pgbd): Identification of genes for lithium response in a prospective sample. *BMC psychiatry* **16**, 129 (2016).

95. Fass. Lithionit. https://www.fass.se/LIF/product?userType=0&nplId=19971114000058.

96. Treasure, J. *et al.* Anorexia nervosa. *Nature reviews. Disease primers* **1**, 15074 (2015).

97. Sattler, F. A., Eickmeyer, S. & Eisenkolb, J. Body image disturbance in children and adolescents with anorexia nervosa and bulimia nervosa: A systematic review. *Eating and weight disorders : EWD* (2019) doi:10.1007/s40519-019-00725-5.

98. American Psychiatric Association. Anorexia Nervosa. in *Diagnostic and statistical manual of mental disorders, fifth edition* (2013). doi:http://dx.doi.org/10.1176/appi.books.9780890425596.dsm04.

99. Støving, R. K., Andries, A., Brixen, K., Bilenberg, N. & Hørder, K. Gender differences in outcome of eating disorders: A retrospective cohort study. *Psychiatry research* **186**, 362–366 (2011).

100. Walsh, B. T. The enigmatic persistence of anorexia nervosa. *American Journal of Psychiatry* **170**, 477–484 (2013).

101. Frank, G. K. What causes eating disorders, and what do they cause? *Biological Psychiatry* **77**, 602–603 (2015).

102. Zipfel, S., Giel, K. E., Bulik, C. M., Hay, P. & Schmidt, U. Anorexia nervosa: Aetiology, assessment, and treatment. *The lancet psychiatry* **2**, 1099–1111 (2015).

103. Swanson, S. A. *et al.* Assessing eating disorder symptoms in adolescence: Is there a role for multiple informants? *International Journal of Eating Disorders* **47**, 475–482 (2014).

104. Stice, E., Marti, C. N., Shaw, H. & Jaconis, M. An 8-year longitudinal study of the natural history of threshold, subthreshold, and partial eating disorders from a community sample of adolescents. *Journal of Abnormal Psychology* **118**, 587–597 (2009).

105. Keski-Rahkonen, A. *et al.* Epidemiology and course of anorexia nervosa in the community. *The American journal of psychiatry* **164**, 1259–1265 (2007).

106. Wade, T. D., Bergin, J. L., Tiggemann, M., Bulik, C. M. & Fairburn, C. G. Prevalence and long-term course of lifetime eating disorders in an adult australian twin cohort. *Australian & New Zealand Journal of Psychiatry* **40**, 121–128 (2006).

107. Bulik, C. M. *et al.* Prevalence, heritability, and prospective risk factors for anorexia nervosa. *Archives of General Psychiatry* **63**, 305 (2006).

108. Raevuori, A. *et al.* Epidemiology of anorexia nervosa in men: A nationwide study of finnish twins. *PloS one* **4**, e4402 (2009).

109. Son, G. E. van, Hoeken, D. van, Bartelds, A. I. M., Furth, E. F. van & Hoek, H. W. Time trends in the incidence of eating disorders: A primary care study in the netherlands. *International Journal of Eating Disorders* **39**, 565–569 (2006).

110. Steinhausen, H.-C. & Jensen, C. M. Time trends in lifetime incidence rates of first-time diagnosed anorexia nervosa and bulimia nervosa across 16 years in a danish nationwide psychiatric registry study. *International Journal of Eating Disorders* **48**, 845–850 (2015).

111. Javaras, K. N. *et al.* Sex- and age-specific incidence of healthcare-register-recorded eating disorders in the complete swedish 1979-2001 birth cohort. *International Journal of Eating Disorders* **48**, 1070–1081 (2015).

112. Yao, S. *et al.* Familial liability for eating disorders and suicide attempts. *JAMA Psychiatry* **73**, 284 (2016).

113. Swanson, S. A. Prevalence and correlates of eating disorders in adolescents. *Archives of General Psychiatry* **68**, 714 (2011).

114. Fernandez-Aranda, F. *et al.* Symptom profile of major depressive disorder in women with eating disorders. *Australian & New Zealand Journal of Psychiatry* **41**, 24–31 (2007).

115. Swinbourne, J. M. & Touyz, S. W. The co-morbidity of eating disorders and anxiety disorders: A review. *European Eating Disorders Review* **15**, 253–274 (2007).

116. Godart, N. T., Flament, M. F., Perdereau, F. & Jeammet, P. Comorbidity between eating disorders and anxiety disorders: A review. *International Journal of Eating Disorders* **32**, 253–270 (2002).

117. Kaye, W. H., Bulik, C. M., Thornton, L., Barbarich, N. & and, K. M. Comorbidity of anxiety disorders with anorexia and bulimia nervosa. *American Journal of Psychiatry* **161**, 2215–2221 (2004).

118. Salbach-Andrae, H. *et al.* Psychiatric comorbidities among female adolescents with anorexia nervosa. *Child Psychiatry and Human Development* **39**, 261–272 (2007).

119. Cederlöf, M. *et al.* Etiological overlap between obsessive-compulsive disorder and anorexia nervosa: A longitudinal cohort, multigenerational family and twin study. *World Psychiatry* **14**, 333–338 (2015).

120. Root, T. L. *et al.* Substance use disorders in women with anorexia nervosa. *International Journal of Eating Disorders* NA–NA (2009) doi:10.1002/eat.20670.

121. Baron-Cohen, S. *et al.* Do girls with anorexia nervosa have elevated autistic traits? *Molecular autism* **4**, 24 (2013).

122. Koch, S. V. *et al.* Autism spectrum disorder in individuals with anorexia nervosa and in their first- and second-degree relatives: Danish nationwide register-based cohort-study. *British Journal of Psychiatry* **206**, 401–407 (2015).

123. Chesney, E., Goodwin, G. M. & Fazel, S. Risks of all-cause and suicide mortality in mental disorders: A meta-review. *World Psychiatry* **13**, 153–160 (2014).

124. Arcelus, J. Mortality rates in patients with anorexia nervosa and other eating disorders. *Archives of General Psychiatry* **68**, 724 (2011).

125. Eddy, K. T. *et al.* Recovery from anorexia nervosa and bulimia nervosa at 22-year follow-up. *The Journal of Clinical Psychiatry* **78**, 184–189 (2016).

126. Schaumberg, K. *et al.* The science behind the academy for eating disorders' nine truths about eating disorders. *European eating disorders review : the journal of the Eating Disorders Association* **25**, 432–450 (2017).

127. Couturier, J. & Lock, J. What is recovery in adolescent anorexia nervosa? *International Journal of Eating Disorders* **39**, 550–555 (2006).

128. Ahren-Moonga, J., Silverwood, R., Klinteberg, B. a. & Koupil, I. Association of higher parental and grandparental education and higher school grades with risk of hospitalization for eating disorders

in females: The uppsala birth cohort multigenerational study. *American Journal of Epidemiology* **170**, 566–575 (2009).

129. Lang, K., Lopez, C., Stahl, D., Tchanturia, K. & Treasure, J. Central coherence in eating disorders: An updated systematic review and meta-analysis. *The World Journal of Biological Psychiatry* **15**, 586–598 (2014).

130. Lavender, J. M. *et al.* Dimensions of emotion dysregulation in anorexia nervosa and bulimia nervosa: A conceptual review of the empirical literature. *Clinical Psychology Review* **40**, 111–122 (2015).

131. Wu, M., Hartmann, M., Skunde, M., Herzog, W. & Friederich, H.-C. Inhibitory control in bulimic-type eating disorders: A systematic review and meta-analysis. *PLoS ONE* **8**, e83412 (2013).

132. Limburg, K., Watson, H. J., Hagger, M. S. & Egan, S. J. The relationship between perfectionism and psychopathology: A meta-analysis. *Journal of Clinical Psychology* **73**, 1301–1326 (2016).

133. O'Hara, C. B., Campbell, I. C. & Schmidt, U. A reward-centred model of anorexia nervosa: A focussed narrative review of the neurological and psychophysiological literature. *Neuroscience & Biobehavioral Reviews* **52**, 131–152 (2015).

134. Karpowicz, E., Skärsäter, I. & Nevonen, L. Self-esteem in patients treated for anorexia nervosa. *International Journal of Mental Health Nursing* **18**, 318–325 (2009).

135. Steinhausen, H.-C., Jakobsen, H., Helenius, D., Munk-Jørgensen, P. & Strober, M. A nation-wide study of the family aggregation and risk factors in anorexia nervosa over three generations. *International Journal of Eating Disorders* **48**, 1–8 (2014).

136. Strober, M. Controlled family study of anorexia nervosa and bulimia nervosa: Evidence of shared liability and transmission of partial syndromes. *American Journal of Psychiatry* **157**, 393–401 (2000).

137. Klump, K. L., Miller, K. B., Keel, P. K., McGue, M. & Iacono, W. G. Genetic and environmental influences on anorexia nervosa syndromes in a populationbased twin sample. *Psychological Medicine* **31**, 737–740 (2001).

138. Bulik, C. M. *et al.* Understanding the relation between anorexia nervosa and bulimia nervosa in a swedish national twin sample. *Biological Psychiatry* **67**, 71–77 (2010).

139. Bulik, C., Yilmaz, Z. & HArdaway, A. Genetics and epigenetics of eating disorders. *Advances in Genomics and Genetics* 131 (2015) doi:10.2147/agg.s55776.

140. Duncan, L. *et al.* Significant locus and metabolic genetic correlations revealed in genome-wide association study of anorexia nervosa. *American journal of psychiatry* **174**, 850–858 (2017).

141. Stelzer, G. *et al.* The genecards suite: From gene data mining to disease genome sequence analyses. *Current protocols in bioinformatics* **54**, 1–30 (2016).

142. Rathjen, T. *et al.* Regulation of body weight and energy homeostasis by neuronal cell adhesion molecule 1. *Nature Neuroscience* **20**, 1096–1103 (2017).

143. Bacon, C. *et al.* Brain-specific foxp1 deletion impairs neuronal development and causes autistic-like behaviour. *Molecular psychiatry* **20**, 632–639 (2015).

144. Sild, M. & Booij, L. Histone deacetylase 4 (HDAC4): A new player in anorexia nervosa? *Molecular Psychiatry* **24**, 1425–1434 (2019).

145. Van den Eynde, F. *et al.* Structural magnetic resonance imaging in eating disorders: A systematic review of voxel-based morphometry studies. *European eating disorders review : the journal of the Eating Disorders Association* **20**, 94–105 (2012).

146. Titova, O. E., Hjorth, O. C., Schiöth, H. B. & Brooks, S. J. Anorexia nervosa is linked to reduced brain structure in reward and somatosensory regions: A meta-analysis of vbm studies. *BMC psychiatry* **13**, 110 (2013).

147. Frank, G. K. W. Recent advances in neuroimaging to model eating disorder neurobiology. *Current psychiatry reports* **17**, 559 (2015).

148. King, J. A., Frank, G. K. W., Thompson, P. M. & Ehrlich, S. Structural neuroimaging of anorexia nervosa: Future directions in the quest for mechanisms underlying dynamic alterations. *Biological psychiatry* **83**, 224–234 (2018).

149. Seitz, J., Herpertz-Dahlmann, B. & Konrad, K. Brain morphological changes in adolescent and adult patients with anorexia nervosa. *Journal of neural transmission (Vienna, Austria : 1996)* **123**, 949–959 (2016).

150. Martin Monzon, B., Hay, P., Foroughi, N. & Touyz, S. White matter alterations in anorexia nervosa: A systematic review of diffusion tensor imaging studies. *World journal of psychiatry* **6**, 177–186 (2016).

151. King, J. A. *et al.* Global cortical thinning in acute anorexia nervosa normalizes following long-term weight restoration. *Biological psychiatry* **77**, 624–632 (2015).

152. Roberto, C. A. *et al.* Brain tissue volume changes following weight gain in adults with anorexia nervosa. *The International journal of eating disorders* **44**, 406–411 (2011).

153. Bernardoni, F. *et al.* Weight restoration therapy rapidly reverses cortical thinning in anorexia nervosa: A longitudinal study. *NeuroImage* **130**, 214–222 (2016).

154. Nickel, K. *et al.* White matter abnormalities in the corpus callosum in acute and recovered anorexia nervosa patients-a diffusion tensor imaging study. *Frontiers in psychiatry* **10**, 490 (2019).

155. Walton, E. *et al.* Exploration of shared genetic architecture between subcortical brain volumes and anorexia nervosa. *Molecular Neurobiology* **56**, 5146–5156 (2018).

156. Steward, T., Menchon, J. M., Jiménez-Murcia, S., Soriano-Mas, C. & Fernandez-Aranda, F. Neural network alterations across eating disorders: A narrative review of fMRI studies. *Current Neuropharmacology* **16**, 1150–1163 (2018).

157. Lawson, E. A. & Klibanski, A. Endocrine abnormalities in anorexia nervosa. *Nature clinical practice. Endocrinology & metabolism* **4**, 407–414 (2008).

158. Misra, M. *et al.* Alterations in cortisol secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *The Journal of Clinical Endocrinology & Metabolism* **89**, 4972–4980 (2004).

159. Misra, M. *et al.* Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *The Journal of Clinical Endocrinology & Metabolism* **88**, 5615–5623 (2003).

160. Croxson, M. S. & Ibbertson, H. K. Low serum triiodothyronine (t3) and hypothyroidism in anorexia nervosa. *The Journal of Clinical Endocrinology & Metabolism* **44**, 167–174 (1977).

161. Grinspoon, S. *et al.* Serum leptin levels in women with anorexia nervosa. *The Journal of Clinical Endocrinology & Metabolism* **81**, 3861–3863 (1996).

162. Misra, M. *et al.* Secretory dynamics of leptin in adolescent girls with anorexia nervosa and healthy adolescents. *American Journal of Physiology-Endocrinology and Metabolism* **289**, E373–E381 (2005).

163. Steinman, J. & Shibli-Rahhal, A. Anorexia nervosa and osteoporosis: Pathophysiology and treatment. *Journal of bone metabolism* **26**, 133–143 (2019).

164. Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. & Kelley, K. W. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature reviews neuroscience* **9**, 46 (2008).

165. Gautron, L. & Layé, S. Neurobiology of inflammation-associated anorexia. *Frontiers in neuroscience* **3**, 3 (2010).

166. Netea, M. G. *et al.* Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nature medicine* **12**, 650 (2006).

167. Heredia, F. P. de, Gómez-Martı'nez, S. & Marcos, A. Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society* **71**, 332–338 (2012).

168. Gibson, D. & Mehler, P. S. Anorexia nervosa and the immune system—a narrative review. *Journal of clinical medicine* **8**, 1915 (2019).

169. Pape, K., Tamouza, R., Leboyer, M. & Zipp, F. Immunoneuropsychiatry—novel perspectives on brain disorders. *Nature Reviews Neurology* 1 (2019).

170. Hedman, A. *et al.* Bidirectional relationship between eating disorders and autoimmune diseases. *Journal of Child Psychology and Psychiatry* **60**, 803–812 (2019).

171. Fetissov, S. O. & Hökfelt, T. On the origin of eating disorders: Altered signaling between gut microbiota, adaptive immunity and the brain melanocortin system regulating feeding behavior. *Current Opinion in Pharmacology* **48**, 82–91 (2019).

172. Dalton, B. *et al.* A meta-analysis of cytokine concentrations in eating disorders. *Journal of psychiatric research* **103**, 252–264 (2018).

173. Negraes, P. D. *et al.* Modeling anorexia nervosa: Transcriptional insights from human iPSC-derived neurons. *Translational psychiatry* **7**, e1060 (2017).

174. Schalla, M. A. & Stengel, A. Activity based anorexia as an animal model for anorexia nervosaA systematic review. *Frontiers in Nutrition* **6**, (2019).

175. Siegfried, Z., Berry, E. M., Hao, S. & Avraham, Y. Animal models in the investigation of anorexia. *Physiology & Behavior* **79**, 39–45 (2003).

176. Nilsson, I. A. The anx/anx mouse–a valuable resource in anorexia nervosa research. *Frontiers in neuroscience* **13**, 59 (2019).

177. Maltais, L. J., Lane, P. W. & Beamer, W. G. Anorexia, a recessive mutation causing starvation in preweanling mice. *Journal of Heredity* **75**, 468–472 (1984).

178. Steinhausen, H.-C. Outcome of eating disorders. *Child and Adolescent Psychiatric Clinics of North America* **18**, 225–242 (2009).

179. Claudino, A. M. *et al.* Antidepressants for anorexia nervosa. *Cochrane Database of Systematic Reviews* (2006) doi:10.1002/14651858.cd004365.pub2.

180. Vos, J. de *et al.* Meta analysis on the efficacy of pharmacotherapy versus placebo on anorexia nervosa. *Journal of Eating Disorders* **2**, (2014).

181. McElroy, S. L. *et al.* Prevalence and correlates of DSM-5 eating disorders in patients with bipolar disorder. *Journal of Affective Disorders* **191**, 216–221 (2016).

182. Tseng, M.-C. M., Chang, C.-H., Chen, K.-Y., Liao, S.-C. & Chen, H.-C. Prevalence and correlates of bipolar disorders in patients with eating disorders. *Journal of Affective Disorders* **190**, 599–606 (2016).

183. McElroy, S. L. *et al.* Prevalence and correlates of eating disorders in 875 patients with bipolar disorder. *Journal of Affective Disorders* **128**, 191–198 (2011).

184. Crow, S. *et al.* Factor analysis of the eating disorder diagnostic scale in individuals with bipolar disorder. *Eating Behaviors* **33**, 30–33 (2019).

185. McElroy, S. L., Kotwal, R., Keck, P. E. & Akiskal, H. S. Comorbidity of bipolar and eating disorders: Distinct or related disorders with shared dysregulations? *Journal of Affective Disorders* **86**, 107–127 (2005).

186. McAulay, C., Hay, P., Mond, J. & Touyz, S. Eating disorders, bipolar disorders and other mood disorders: Complex and under-researched relationships. *Journal of Eating Disorders* **7**, (2019).

187. Chen, Y., Bidwell, L. C. & Norton, D. Trait vs. State markers for schizophrenia: Identification and characterization through visual processes. *Current Psychiatry Reviews* **2**, 431–438 (2006).

188. Scott, J. *et al.* An examination of the quality and performance of the alda scale for classifying lithium response phenotypes. *Bipolar disorders* (2019).

189. Hällström, T., Damström Thakker, K., Forsell, Y., Lundberg, I. & Tinghög, P. The part study: A population based study of mental health in the stockholm county: Study design phase l (1998-2000). *Stockhom, Sweden: The PART Study Group* (2003).

190. Young, R. C., Biggs, J. T., Ziegler, V. E. & Meyer, D. A. A rating scale for mania: Reliability, validity and sensitivity. *The British journal of psychiatry : the journal of mental science* **133**, 429–435 (1978).

191. First, M. B., Spitzer, R. L., Gibbon, M. & Williams, J. B. *User's guide for the structured clinical interview for dsm-iv axis i disorders scid-i: Clinician version.* (American Psychiatric Pub, 1997).

192. Thornton, L. M. *et al.* The anorexia nervosa genetics initiative (angi): Overview and methods. *Contemporary clinical trials* **74**, 61–69 (2018).

193. Bixo, L., Cunningham, J. L., Ekselius, L., Öster, C. & Ramklint, M. 'Sick and tired': Patients reported reasons for not participating in clinical psychiatric research. *Health Expectations* (2019) doi:10.1111/hex.12977.

194. Sinnwell, J. & Schaid, D. *Haplo.stats: Statistical analysis of haplotypes with traits and covariates when linkage phase is ambiguous.* (2016).

195. Purcell, S. *et al.* PLINK: A tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics* **81**, 559–575 (2007).

196. Coleman, J. R. I. *et al.* Quality control, imputation and analysis of genome-wide genotyping data from the illumina humancoreexome microarray. *Briefings in functional genomics* **15**, 298–304 (2016).

197. International HapMap Consortium. A haplotype map of the human genome. *Nature* **437**, 1299–1320 (2005).

198. Dudbridge, F. & Gusnanto, A. Estimation of significance thresholds for genomewide association scans. *Genetic epidemiology* **32**, 227–234 (2008).

199. International HapMap Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature* **467**, 52–58 (2010).

200. 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).

201. Howie, B. N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS genetics* **5**, e1000529 (2009).

202. Leeuw, C. A. de, Mooij, J. M., Heskes, T. & Posthuma, D. MAGMA: Generalized gene-set analysis of gwas data. *PLoS computational biology* **11**, e1004219 (2015).

203. Thomas, P. D. *et al.* PANTHER: A library of protein families and subfamilies indexed by function. *Genome research* **13**, 2129–2141 (2003).

204. Watanabe, K., Taskesen, E., Bochoven, A. van & Posthuma, D. Functional mapping and annotation of genetic associations with fuma. *Nature communications* **8**, 1826 (2017).

205. Buonocore, M. H. & Maddock, R. J. Magnetic resonance spectroscopy of the brain: A review of physical principles and technical methods. *Reviews in the neurosciences* **26**, 609–632 (2015).

206. Li, C.-T., Yang, K.-C. & Lin, W.-C. Glutamatergic dysfunction and glutamatergic compounds for major psychiatric disorders: Evidence from clinical neuroimaging studies. *Frontiers in psychiatry* **9**, 767 (2018).

207. Ramadan, S., Lin, A. & Stanwell, P. Glutamate and glutamine: A review of in vivo mrs in the human brain. *NMR in biomedicine* **26**, 1630–1646 (2013).

208. Hancu, I. & Port, J. The case of the missing glutamine. *NMR in biomedicine* **24**, 529–535 (2011).

209. Hurd, R. *et al.* Measurement of brain glutamate using te-averaged press at 3T. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* **51**, 435–440 (2004).

210. Port, J. D., Unal, S. S., Mrazek, D. A. & Marcus, S. M. Metabolic alterations in medication-free patients with bipolar disorder: A 3T csf-corrected magnetic resonance spectroscopic imaging study. *Psychiatry research* **162**, 113–121 (2008).

211. OLINK proteomics. *PEA: An enabling technology for highmultiplex protein biomarker discovery.* https://www.olink.com/content/uploads/2017/12/1074-v1.0-PEA-White-paper_final.pdf (2017).

212. OLINK proteomics. *Data normalization and standardization.* https://www.olink.com/content/uploads/2018/05/Data-normalization-and-standardization_v1.0.pdf (2018).

213. OLINK proteomics. *Measuring protein biomarkers with olink — technical comparisons and orthogonal validation.* https://www.olink.com/content/uploads/2019/06/Olink-technical-comparisons-and-orthogonal-validation-v1.0.pdf (2019).

214. Vangipuram, M., Ting, D., Kim, S., Diaz, R. & Schüle, B. Skin punch biopsy explant culture for derivation of primary human fibroblasts. *JoVE (Journal of Visualized Experiments)* e3779 (2013).

215. Diecke, S. *et al.* Novel codon-optimized mini-intronic plasmid for efficient, inexpensive and xeno-free induction of pluripotency. *Scientific Reports* **5**, (2015).

216. Ly, A., Etz, A., Marsman, M. & Wagenmakers, E.-J. Replication bayes factors from evidence updating. *Behavior research methods* **51**, 2498–2508 (2019).

217. Simonsohn, U. Small telescopes: Detectability and the evaluation of replication results. *Psychological science* **26**, 559–569 (2015).

218. Dumas-Mallet, E., Button, K. S., Boraud, T., Gonon, F. & Munafò, M. R. Low statistical power in biomedical science: A review of three human research domains. *Royal Society open science* **4**, 160254 (2017).

219. Matheson, G. J. Reliability, replicability and reproducibility in pet imaging. (Karolinska Institutet, 2018).

220. Probabilistic World. Frequentist and bayesian approaches in statistics. https://www.probabilisticworld.com/frequentist-bayesian-approaches-inferential-statistics/ (2016).

221. McElreath, R. *Statistical rethinking: A bayesian course with examples in r and stan.* (Chapman; Hall/CRC, 2018).

222. Kass, R. E. & Raftery, A. E. Bayes factors. *Journal of the American Statistical Association* **90**, 773–795 (1995).

223. Jamil, T. *et al.* Default 'gunel and dickey' bayes factors for contingency tables. *Behavior Research Methods* **49**, 638–652 (2017).

224. JASP Team. JASP (Version 0.11.1)[Computer software]. (2019).

225. UCLA: Statistical Consulting Group. Introduction to linear mixed models. https://stats.idre.ucla.edu/other/mult-pkg/introduction-to-linear-mixed-models/ (2020).

226. McElreath, R. Multilevel regression as default. https://elevanth.org/blog/2017/08/24/multilevel-regression-as-default/ (2017).

227. Hajduk, G. K. Introduction to linear mixed models. https://ourcodingclub.github.io/2017/03/15/mixed-models.html/ (2017).

228. Gelman, A. & Hill, J. *Data analysis using regression and multilevel/hierarchical models.* (Cambridge University Press, 2007).

229. Lyons, M. Generalised additive models (gams): An introduction. http://environmentalcomputing.net/intro-to-gams/ (2018).

230. Pedersen, E. J., Miller, D. L., Simpson, G. L. & Ross, N. Hierarchical generalized additive models: An introduction with mgcv. *PeerJ Preprints* **6**, e27320v1 (2018).

231. Flom, P. An introduction to quantile regression. https://towardsdatascience.com/an-introduction-to-quantile-regression-eca5e3e2036a (2018).

232. Koenker, R. *Quantile regression in r: A vignette.* (2019).

233. Baum, C. F. Quantile regression (ec 823: Applied econometrics). http://fmwww.bc.edu/EC-C/S2013/823/EC823.S2013.nn04.slides.pdf (2013).

234. Koenker, R. *Quantreg: Quantile regression.* (2019).

235. Manning, B. D. & Toker, A. AKT/pkb signaling: Navigating the network. *Cell* **169**, 381–405 (2017).

236. Howell, K. R. & Law, A. J. Neurodevelopmental concepts of schizophrenia in the genome-wide association era: AKT/mTOR signaling as a pathological mediator of genetic and environmental programming during development. *Schizophrenia Research* (2019).

237. Freyberg, Z., Ferrando, S. J. & Javitch, J. A. Roles of the akt/gsk-3 and wnt signaling pathways in schizophrenia and antipsychotic drug action. *American Journal of Psychiatry* **167**, 388–396 (2009).

238. Toyota, T., Yamada, K., Detera-Wadleigh, S. D. & Yoshikawa, T. Analysis of a cluster of polymorphisms in akt1 gene in bipolar pedigrees: A family-based association study. *Neuroscience letters* **339**, 5–8 (2003).

239. Karege, F. *et al.* Association of akt1 gene variants and protein expression in both schizophrenia and bipolar disorder. *Genes, Brain and Behavior* **9**, 503–511 (2010).

240. Karege, F. *et al.* Genetic overlap between schizophrenia and bipolar disorder: A study with akt1 gene variants and clinical phenotypes. *Schizophrenia research* **135**, 8–14 (2012).

241. Disorder, B., Psychiatric Genomics Consortium. Electronic address: douglas.ruderfer@vanderbilt.edu, S. W. G. of the, Disorder, B. & Psychiatric Genomics Consortium, S. W. G. of the. Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. *Cell* **173**, 1705–1715.e16 (2018).

242. Lau, A. & Tymianski, M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Archiv : European journal of physiology* **460**, 525–542 (2010).

243. McCullumsmith, R. E. *et al.* Decreased nr1, nr2a, and sap102 transcript expression in the hippocampus in bipolar disorder. *Brain research* **1127**, 108–118 (2007).

244. Beneyto, M., Kristiansen, L. V., Oni-Orisan, A., McCullumsmith, R. E. & Meador-Woodruff, J. H. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **32**, 1888–1902 (2007).

245. Beneyto, M. & Meador-Woodruff, J. H. Lamina-specific abnormalities of nmda receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **33**, 2175–2186 (2008).

246. Rao, J. S., Harry, G. J., Rapoport, S. I. & Kim, H. W. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Molecular psychiatry* **15**, 384–392 (2010).

247. Rathje, M. *et al.* Genetic variants in the bipolar disorder risk locus syne1 that affect cpg2 expression and protein function. *Molecular psychiatry* (2019) doi:10.1038/s41380-018-0314-z.

248. Luykx, J. *et al.* Region and state specific glutamate downregulation in major depressive disorder: A meta-analysis of 1H-mrs findings. *Neuroscience & Biobehavioral Reviews* **36**, 198–205 (2012).

249. Arnone, D., Mumuni, A. N., Jauhar, S., Condon, B. & Cavanagh, J. Indirect evidence of selective glial involvement in glutamate-based mechanisms of mood regulation in depression: Meta-analysis of absolute prefrontal neuro-metabolic concentrations. *European Neuropsychopharmacology* **25**, 1109–1117 (2015).

250. Wirth, C. *et al.* DTNBP1 (dysbindin) gene variants: In vivo evidence for effects on hippocampal glutamate status. *Current pharmaceutical biotechnology* **13**, 1513–1521 (2012).

251. Rogdaki, M. *et al.* Glutamatergic function in a genetic high-risk group for psychosis: A proton magnetic resonance spectroscopy study in individuals with 22q11. 2 deletion. *European Neuropsychopharmacology* (2019).

252. Legind, C. S. *et al.* Heritability of cerebral glutamate levels and their association with schizophrenia spectrum disorders: A 1 [h]-spectroscopy twin study. *Neuropsychopharmacology* **44**, 581 (2019).

253. Soeiro-de-Souza, M. G. *et al.* Bcl-2 rs956572 polymorphism is associated with increased anterior cingulate cortical glutamate in euthymic bipolar i disorder. *Neuropsychopharmacology* **38**, 468 (2013).

254. Gruber, O. *et al.* Association of the brain-derived neurotrophic factor val66met polymorphism with magnetic resonance spectroscopic markers in the human hippocampus: In vivo evidence for effects on the glutamate system. *European archives of psychiatry and clinical neuroscience* **262**, 23–31 (2012).

255. Ponta, H., Sherman, L. & Herrlich, P. A. CD44: From adhesion molecules to signalling regulators. *Nature reviews Molecular cell biology* **4**, 33 (2003).

256. Johnson, P. & Ruffell, B. CD44 and its role in inflammation and inflammatory diseases. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)* **8**, 208–220 (2009).

257. Dzwonek, J. & Wilczynski, G. M. CD44: Molecular interactions, signaling and functions in the nervous system. *Frontiers in cellular neuroscience* **9**, 175 (2015).

258. Liu, Y. *et al.* CD44 expression identifies astrocyte-restricted precursor cells. *Developmental biology* **276**, 31–46 (2004).

259. Matzke, A. *et al.* Haploinsufficiency of c-met in cd44-/- mice identifies a collaboration of cd44 and c-met in vivo. *Molecular and cellular biology* **27**, 8797–8806 (2007).

260. Michael, N., Erfurth, A. & Pfleiderer, B. Elevated metabolites within dorsolateral prefrontal cortex in rapid cycling bipolar disorder. *Psychiatry Research: Neuroimaging* **172**, 78–81 (2009).

261. Charles, B. Population pharmacokinetics: An overview. *Aust Prescr* **37**, 210–3 (2014).

262. Ette, E. I. & Williams, P. J. Population pharmacokinetics ii: Estimation methods. *The Annals of pharmacotherapy* **38**, 1907–1915 (2004).

263. Serra-Juhe, C. *et al.* Novel genes involved in severe early-onset obesity revealed by rare copy number and sequence variants. *PLoS genetics* **13**, e1006657 (2017).

264. Barbitoff, Y. *et al.* Identification of novel candidate markers of type 2 diabetes and obesity in russia by exome sequencing with a limited sample size. *Genes* **9**, 415 (2018).

265. Mahajan, A. *et al.* Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nature genetics* **50**, 1505 (2018).

266. Kichaev, G. *et al.* Leveraging polygenic functional enrichment to improve gwas power. *American journal of human genetics* **104**, 65–75 (2019).

267. Hübel, C. *et al.* Genomics of body fat percentage may contribute to sex bias in anorexia nervosa. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **180**, 428–438 (2019).

268. Chang, W., Cheng, J., Allaire, J., Xie, Y. & McPherson, J. Shiny: Web application framework for r. (2019).

269. Masters, C. *et al.* Alzheimer's disease. *Nature reviews Disease primers* **1**, 794 (2015).

270. Bos, M. A. van den, Geevasinga, N., Higashihara, M., Menon, P. & Vucic, S. Pathophysiology and diagnosis of als: Insights from advances in neurophysiological techniques. *International Journal of Molecular Sciences* **20**, 2818 (2019).

271. Poewe, W. *et al.* Parkinson disease. *Nature reviews Disease primers* **3**, 17013 (2017).

272. Friese, M. A., Schattling, B. & Fugger, L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nature reviews. Neurology* **10**, 225–238 (2014).

273. Gaetani, L. *et al.* Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry* jnnp–2018 (2019).

274. Khalil, M. *et al.* Neurofilaments as biomarkers in neurological disorders. *Nature reviews. Neurology* **14**, 577–589 (2018).

275. Teunissen, C. E. & Khalil, M. Neurofilaments as biomarkers in multiple sclerosis. *Multiple sclerosis (Houndmills, Basingstoke, England)* **18**, 552–556 (2012).

276. Kuhle, J. *et al.* A comparative study of csf neurofilament light and heavy chain protein in ms. *Multiple Sclerosis Journal* **19**, 1597–1603 (2013).

277. Mattsson, N., Andreasson, U., Zetterberg, H. & Blennow, K. Association of plasma neurofilament light with neurodegeneration in patients with alzheimer disease. *JAMA neurology* **74**, 557–566 (2017).

278. Alcolea, D. *et al.* CSF sAPPβ, ykl-40, and neurofilament light in frontotemporal lobar degeneration. *Neurology* **89**, 178–188 (2017).

279. Shahim, P. *et al.* Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. *Scientific reports* **6**, 36791 (2016).

280. Jakobsson, J. *et al.* Elevated concentrations of neurofilament light chain in the cerebrospinal fluid of bipolar disorder patients. *Neuropsychopharmacology* **39**, 2349 (2014).

281. Disanto, G. *et al.* Serum neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Annals of neurology* **81**, 857–870 (2017).

282. Shahim, P., Zetterberg, H., Tegner, Y. & Blennow, K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology* **88**, 1788–1794 (2017).

283. Kalm, M. *et al.* Serum concentrations of the axonal injury marker neurofilament light protein are not influenced by blood-brain barrier permeability. *Brain research* **1668**, 12–19 (2017).

284. Ehrlich, S. *et al.* S100B in underweight and weight-recovered patients with anorexia nervosa. *Psychoneuroendocrinology* **33**, 782–788 (2008).

285. Ehrlich, S. *et al.* Glial and neuronal damage markers in patients with anorexia nervosa. *Journal of neural transmission (Vienna, Austria : 1996)* **115**, 921–927 (2008).

286. Yang, L. L. *et al.* Enteric short-chain fatty acids promote proliferation of human neural progenitor cells. *Journal of Neurochemistry* (2019) doi:10.1111/jnc.14928.

287. Open Science Collaboration. Estimating the reproducibility of psychological science. *Science* **349**, aac4716–aac4716 (2015).

288. Baker, M. 1,500 scientists lift the lid on reproducibility. *Nature News Feature* (2016).

289. Amrhein, V., Greenland, S. & McShane, B. Scientists rise up against statistical significance. (2019).

290. Blacker, C. J. *et al.* EAAT2 as a research target in bipolar disorder and unipolar depression: A systematic review. *Molecular Neuropsychiatry* 1–16 (2019) doi:10.1159/000501885.

291. Woolley, A. W., Chabris, C. F., Pentland, A., Hashmi, N. & Malone, T. W. Evidence for a collective intelligence factor in the performance of human groups. *Science* **330**, 686–688 (2010).

292. Credé, M. & Howardson, G. The structure of group task performanceA second look at "collective intelligence": Comment on woolley et al. (2010). *Journal of Applied Psychology* **102**, 1483–1492 (2017).